Flowering and the Peak N Hypothesis in Annual Plants

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This thesis is dedicated to my daughter, *Léa*,

who changed *everything*.

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« Le petit prince, qui assistait à l'installation d'un bouton énorme, sentait bien qu'il en sortirait une apparition miraculeuse, mais la fleur n'en finissait pas de se préparer à être belle, à l'abri de sa chambre verte. Elle choisissait avec soin ses couleurs. Elle s'habillait lentement, elle ajustait un a un ses pétales. Elle ne voulait pas sortir tout fripée comme les coquelicots. Elle ne voulait apparaître que dans le plein rayonnement de sa beauté. »

Le Petit Prince, *Antoine de Saint-Exupery.*

General Introduction

On the importance of flowering

Flowering plants appeared in the late Triassic (Hochuli and Feist-Burkhardt 2013), and shaped terrestrial life like no other life forms (Melillo *et al.* 1993, Smith *et al.* 2013). Through evolutionary times, angiosperms have developed countless interactions with a wide variety of organisms ranging from microbes (Soto *et al.* 2009, Wang *et al.* 2011), to large mammals (Bryant *et al.* 1983, some now extinct Barlow 2000), although most fascinating interactions take place with insects (Weiblen 2002, Pringle *et al.* 2012). Furthermore, as plant can't escape local conditions due to their sessile lifestyle, they display original adaptations to virtually all terrestrial environments (Humboldt 1805, McNickle and Dybzinski 2013). This large scope of potential interactions make flowering plants an invaluable study object in ecology, point not missed by Darwin who dedicated an extensive amount of work on plants and their reproductive strategies (Darwin 1862, 1876, 1877).

Among angiosperms, annual plants have been selected to complete their lifecycle in one growing season. This short lifecycle appeared as a response to hostile (Cole 1954, Stearns 1976) or stressful environment (Yanovsky and Kay 2003, Wada and Takeno 2010, Kawagoe and Kudoh 2010). Annual lifecycles have driven evolutionary biologists interests further since Cole wrote: "*For an annual species, the absolute gain in intrinsic population growth which could be achieved by changing to the perennial reproductive habit would be exactly equivalent to adding one individual to the average litter size*". It draws from this statement that there must be strong fitness disadvantages of an annual lifecycle not directly taken into account from the reproductive output perspective. And indeed, an annual lifecycle implies that a large part of the plant fitness is

determined by one event: flowering. As a consequence, the timing of flowering is considered under strong selection (Rose *et al.* 2009, Vergeer and Kunin 2011, Zuest *et al.* 2012). To be successful, a plant must possess the ability to respond to environmental and internal cues with high sensibility in order to engage in reproduction at the moment that is most favourable (Dorn *et al.* 2000, de Montaigu *et al.* 2010). The switch from vegetative growth to reproductive growth is sharp (Iwasa and Cohen 1989, Levy and Dean 1998, Melzer *et al.* 2008) and occurs usually only once in an annual plant's life (King and Roughgarden 1982). Flowering time in annual plants is altogether an optimization problem (Cohen 1976), an evolutionary game (Stearns and Koella 1986), a molecular and genetic process (Bernier *et al.* 1993), and an ecological response (Roux *et al.* 2006). The study of timing of flowering timing has been a vivid field in ecology for the past forty years and despite great theoretical and experimental contributions many areas of knowledge remain *terra incognita* (Cerdan and Chory 2003, Melzer *et al.* 2008, Zuest *et al.* 2012, Joseph *et al.* 2013).

On the flowering transition

The greatest advances in understanding of the mechanisms leading to flowering occurred through meticulous examination of plant mutants by molecular biologists (Simpson *et al.* 1999, Harberd 2006). The decision to focus on one plant, *Arabidopsis thaliana*, and to operate mutation on it to silence, overexpress or modify the functioning of genes was at the origin of an impressive number of ground-breaking discoveries in the regulation of flowering in plants (Sheldon *et al.* 2000, Simpson and Dean 2002, Hayama and Coupland 2003, Cerdan and Chory 2003, Stinchcombe *et al.* 2004, Henderson and Dean 2004, Melzer *et al.* 2008). In 2002, Simpson and Dean summarised the work on the genetic basis of flowering timing with the multiple pathways model (Figure.0.1), demonstrating the richness of genes involved and environmental cues the

plant integrates in order to initiate flowering. Researchers now differentiate four main pathways that affects flowering; they are named the temperature (or vernalization) pathway, the photoperiod pathway, the hormonal pathway, and the autonomous pathway. They interact quantitatively with each other to make a plant flower optimally (Boss *et al.* 2004). Recently, Wahl *et al.* (2013) uncovered yet another pathway that relies on accurate detection of a particular sugar (trehalose-6-phosphate) to inform the plant on its nutritional status. Wahl and its colleagues have also demonstrated that ensuring a correct nutritional status of the plant before flowering overrides the four aforementioned pathways.

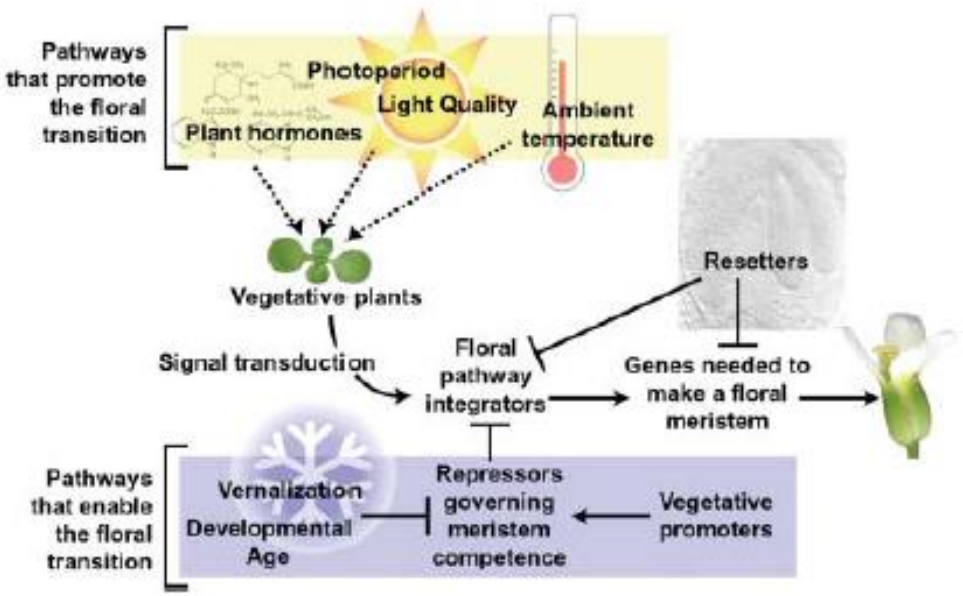

Figure.0.1. (From Boss *et al.* 2004) Pathways that enable or promote the floral transition determine flowering time. The different pathways are grouped in those that promote and those that enable the floral transition. The enabling pathways regulate the ability of the meristem to respond to floral promotive signals from different environmental and endogenous cues.

From a molecular point of view, flowering is a phenomenon actively repressed by the plant (through the expression of *FLC* in temperature-related settings or *CO* in photoperiod-related

ones) until the production of promoters reaches a threshold. The right threshold of expression over repression is obtained through the process of natural selection (Shindo *et al.* 2007). As a consequence, the variation in flowering time among ecotypes of the same species or among different annual plant species is due to the selection for different thresholds under different conditions (Mitchell-Olds 1996, Koornneef *et al.* 1998). Simply put, a plant initiates flowering when internal cues (gene expression) stimulated by external cues (light, temperature, etc…) reaches the appropriate threshold. This approach, though powerful, usually leaves out of the picture two key component of a plant lifecycle: the variability of the environment and the effect the plants have on the environment it is growing in. Indeed, the methodology used in many studies focuses mainly on the variation among plant types in similar settings (Alonso-Blanco *et al.* 1998, 1999, Koornneef *et al.* 1998, Gazzani *et al.* 2003), rarely adopts an approach involving environmental gradients (except for the photoperiod pathway, e.g.: Cerdan and Chory 2003, Wilczek *et al.* 2009), and usually strive to maintain identical conditions throughout the experiment thus masking any impact the plant may have on its environment. This was a perfectly sensible approach of the problem in order to dissect the mechanism behind flowering. However, plants typically grow up in changing conditions (Metcalf *et al.* 2008, Lv *et al.* 2008, Nord and Lynch 2009), experiencing variation in both abiotic and biotic conditions over their lifetime and across generations. Now is the time to compare side by side the recently acquired molecular knowledge on flowering time with the older theoretical body of literature that deals with the evolution of flowering time under various ecological conditions.

On rules for flowering

The study of optimal flowering time is old (Spencer 1884 cited in Zeide 1978). Although, mechanistic models of flowering driven by sets of equations appeared more recently in the 1970s

(Cohen 1971, Paltridge and Denholm 1974). With Cohen, the ecological and evolutionary problem of flowering was for the first time offered a possibly general solution. Succinctly, Cohen's model provides a set of equations that describes the plant allocation process through time as a function of the plant mass and its inherent growth ability. By optimising the mass of the final seed set, Cohen proved that in a predictable environment, an annual plant should flower once and at once and therefore that annual plant should have a two-phase lifecycle separated by a sharp flowering event. During the first part of the lifecycle the plant would only grow vegetative structures while in the second part the plant would only grow reproductive structures. However, in stochastic conditions, his model allows for a transitional period with growth of both vegetative and reproductive structure. This seminal work led to the development of numerous models dealing with most aspects of plant's life-history (Paltridge and Denholm 1974, King and Roughgarden 1982, Iwasa and Cohen 1989, Kudoh *et al.* 2002). The timing of flowering in these models generally boils down to two main rules: the age rule and the size rule.

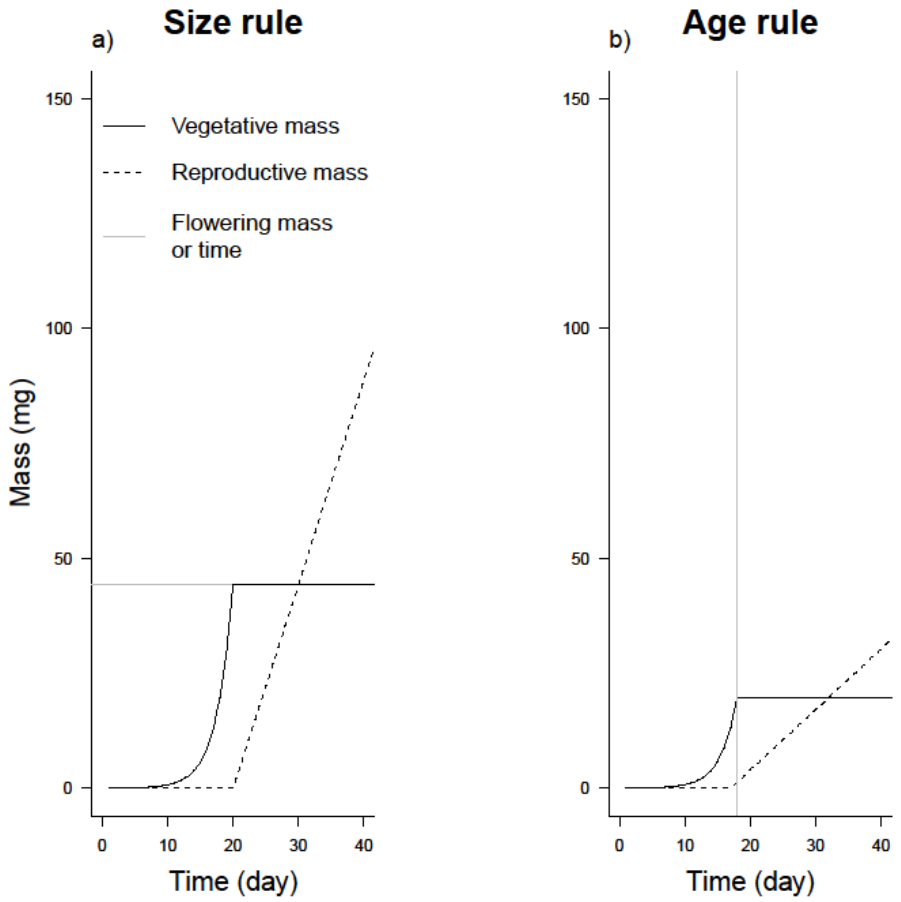

Figure.0.2. a) Size rule and a) age rule for flowering. In the case of an age rule for flowering, the plant grows until a certain age (grey line on day 20) before initiating flowering. As for the size rule, the plant initiates flowering as the plant reaches a certain size (grey line at 10 mg). Growth lines are produced assuming an exponential growth of vegetative tissues and a linear growth of reproductive tissues.

The age rule for flowering implies that a plant grows until it reaches a given age and then initiates flowering; this rule parallels the "competence threshold" (Henderson and Dean 2004) a plant is supposed to acquire before initiating flowering. The size rule implies that a plant cannot flower before it has reached a certain size (Figure.0.2). The "expression threshold" (Satake 2010) used by molecular biologists parallels this idea. Both rules share this idea of an inflexible trigger for flowering determined through evolution. Variation in the size or age at flowering can be

introduced in many of the existing models using stochastic environment in the same fashion as Cohen. But evolutionary models, like genetic ones, insufficiently account for the inherent plasticity of plants in the way they perform their lifecycle and the feedback loops plants may create with the environment they are growing in.

Iwasa (2000) developed a dynamic optimisation model for the growth of plants that deal with this issue. In his model, plant growth and then flowering is determined by the plant environment and the plant physiological ability. Performing a fitness optimisation procedure, this model is able to determine the timing of flowering and the cessation of plant vegetative growth. Unfortunately, Iwasa's model still considers the plant has no effect on the local conditions. Because plants affect the environment they're growing in, and because an appropriate timing of flowering is of prime importance for a plant to successfully pass on its gene, one should develop a model that accounts for these effects.

Inspired by the work of both theoretical biologists and molecular biologists, this new model for plant growth would integrate key processes of the plant lifecycle and how they interact with each other to optimise fitness. The goal of the present work is to create a new model for an annual plant growth that rely on accurate detection of the depletion of a key soil resource, nitrogen, to initiate three processes that a plant must correctly and timely achieve: the balance of roots and leaves growth, the timing of flowering, and the pattern of leaves senescence.

Testing whether a new model for growth and flowering is better than the currently existing ones implies that one needs to compare the different models with data to test how well each model predicts plant's lifecycle

On the method to discriminate rules

Historically, biological models have been separated into two groups: while empirical models focus on finding equations that fit observed data without regard to their biological meaning (Lotka 1920, Pearsall 1927, Yin *et al.* 2003, Paine *et al.* 2012), mechanistic models focus on producing biologically meaningful equations that can explain observations (Paltridge and Denholm 1974, Hunt 1981, Iwasa and Cohen 1989, Thornley 1998). Because their goal is a coherent representation of a biological problem, mechanistic models tend to be rather qualitative than quantitative (Chen and Reynolds 1997, Stoll *et al.* 2002, Damgaard and Weiner 2008), as they prioritise conceptual consistency over accuracy (Reynolds and Chen 1996, West *et al.* 1997). Regardless of the group in which a model falls, its ability to give meaningful predictions or a deeper understanding is what ultimately prevails (Stearns and Koella 1986, Box and Draper 1987, Hubbell 2006), and both empirical and mechanistic models have been successfully used to model the growth of annual plants (Yin *et al.* 2003, Nord *et al.* 2011, Paine *et al.* 2012). The boundary between those two categories is now fading with the rise of Bayesian inference methods that provide ecologists with new ways to quantify parameter values from their models (Chib and Greenberg 1995, Clark 2005). Bayesian modelling gives the opportunity to fit equations on observed data (as empirical models do) using a biologically meaningful set of equations (as mechanistic models do). Using Bayesian inference to test models seems to bring the best of both worlds as it provides parameter values on empirical equations with uncertainty (Smith *et al.* 2013) or allows for model selection across a variety of mechanistic models (Carlin and Chib 1995, Newbold *et al.* 2013, Garcia-Valdes *et al.* 2013).

Outline of the thesis

In this thesis, we define a new rule for flowering. This rule relies on an accurate assessment of the soil nitrogen content that translates into a plant lifecycle integrating environmental and physiological limits to predict different aspects of the plant growth. We then explore theoretical predictions associated with this new rule in a variety of ecological settings: from different fertilization treatments, to biotic interactions such as competition or herbivory. After ensuring our model assumptions are relevant, we finally test the model against a dataset designed to maximise precision of the assessment of flowering timing. The relevance of our new rule for flowering compared to previous one is assessed using a recent probalistic and computational tool: Filzbach. This thesis provides the scientific community with a new model of plant growth that has the following properties: it is 1) conceptually simple and 2) applies potentially to a large variety of abiotic and biotic conditions. From the study of the model properties emerges 3) a new rule to predict flowering in annual plants and 4) an optimal senescence pattern. Finally, the model is 5) designed to allow the integration of ecological constraints in possibly non-ecological settings such as molecular biology. In addition, this thesis contains 6) a recent application of Bayesian tools that offers quantitative predictions together with a user-friendly model-selection device.

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Summary

In the present thesis, I focused on the integration of environmental variables by the plants in order to achieve a successful transition to flowering. This integration is realised through the use of a new model for the growth of an annual plant: the peak N model. The aim of the model is to describe key events of a plant's life in an integrated manner using the plant constant balance between nitrogen uptake and carbon production.

In **Chapter 1**, we lay out the model. We define the assumptions driving the model, mainly that the pool of accessible nitrogen is limited. We then explore the predictions the model provides for a variety of i) environmental changes such as initial nitrogen concentration, pot sizes and the possibility of nitrogen fertilization and ii) physiological changes at the level of the plant such as improved photosynthetic efficiency, uptake efficiency or modified C:N ratios. We show that the model predicts contrasting and counter-intuitive results for the final reproductive mass and time to flower as a function of the changes in the way nitrogen is provided to the plant. We give evidence from the literature that the plant responses predicted by the model are in line with a wide range of observations.

In **Chapter 2**, we explore the assumptions of the model, and particularly how the model assumptions relate to fitness. To be able to give sensible fitness predictions, the model is modified to integrate competitors. The ability to the plant to respond to competitor's density and competitor's strategies is assessed. In particular, we address whether our model genuinely predicts a tragedy of the commons in root competition. We confirm three of the main assumptions of the model. 1) An annual plant must maximise growth rate before flowering. 2) Peak N is an absolute upper limit to the time of flowering and a plant in competition is driven to

flower approximately at peak N. 3) A strategy consisting in investing all means of production to reproduction past peak N is more successful than proposed alternatives. Furthermore, as overallocation to roots does not constitute a better growing strategy, we predict that no tragedy of the commons is expected to occur when plants are grown in competition despite the fact that competition accelerates a plant's lifecycle.

In **Chapter 3**, we explore the flexibility of a plant behaving as predicted by the peak N model in the context of herbivory. We define three main predation types: Leaf-chewing, Root-chewing, and Sap-sucking. We then explore the consequences of the different way a plant experiences predation in terms of fitness and flowering time. We show that the way a plant is consumed has important fitness consequences that our model is able to take into account. Specifically, we show that sap-sucking delays flowering and can be deleterious for the herbivore if too much sap is stolen. A plant is able to withstand high level of leaf or root damage depending on the timing of herbivory. Finally, we show their might exist a limit to using a peak N rule for flowering in the case of extreme events of herbivory. With this chapter, the peak N model offers a new conceptual framework for the study of herbivory in annual plants.

In **Chapter 4**, the model is tested against experimental data. We test the ability of the model to predict growth trajectories, flowering time and senescence pathways for 8 different ecotypes of *Arabidopsis thaliana* grown in a climate chamber. Data show that slight modifications of the model are necessary to accommodate the growth of *Arabidopsis* but that, once corrected, the model predicts plant growth with high accuracy. Using Bayesian methods, we compare the quality of the model's prediction against a variety of alternative models. We showed that a peak N rule for flowering predicted data better than a size- or an age-rule for flowering. Moreover, we

managed to account for differences in the physiological parameters among the ecotypes and therefore predict ecotype-specific growth trajectories and flowering time.

Parts of Chapter 1 and 2 were merged into one article for submission to New Phytologist with the following title “Is ‘Peak N’ key to understanding the timing of flowering in annual plants?”. Chapter 3 and 4 are written as chapters and are expected to be modified into articles for publication. For the purpose of clarity, the basis of the peak N model is reminded in the method section of all chapters and therefore causes some overlapping among chapters.

Dr. Neil Dalchau from the Computational Science Laboratory of Microsoft Research contributed large parts of the conceptual framework of the present thesis and receives due accreditation as author in the chapter in which he contributed.

Zusammenfassung

In der vorliegenden Doktorarbeit konzentrierte ich mich darauf, wie eine Integration von Umweltvariabeln durch Pflanzen erzielt wird, um einen erfolgreichen Übergang zur Blüte zu erreichen. Diese Integration wird mit Hilfe eines neuen Models für das Wachstum einer einjährigen Pflanze betrachtet, dem "Stickstoff-Höhepunkt-Modell". Das Ziel des Modells ist die integrierte Beschreibung von Schlüsselereignissen im Lebenszyklus einer Pflanze durch eine konstante Balance zwischen Stickstoffaufnahme und Kohlenstoffproduktion.

Im **ersten Kapitel** legen wir das Modell aus. Wir definieren die Annahmen welche das Modell beeinflussen, vor allem dass der für die Pflanze erschliessbare Stickstoffpool limitiert ist. Wir erkunden dann die Voraussagen welche das Modell liefert, nämlich für eine Vielfalt von i) Veränderungen der Umwelt, z.B. anfängliche Stickstoffkonzentration, Topfgrösse und die Möglichkeit zur Stickstoffdüngung, und ii) physiologischen Veränderungen auf der Ebene der Pflanze, so z.B. verbesserte Photosynthese-Effizienz, Aufnahme-Effizienz oder veränderte C:N Verhältnisse. Wir zeigen, dass das Modell entgegengesetzte und nicht eingängige Voraussagen macht zur letztendlichen reproduktiven Masse und Blühzeit in Abhängigkeit von Veränderungen der Art des Stickstoffangebotes für die Pflanze. Wir führen Belege aus der Literatur an, welche zeigen, dass die vom Modell vorausgesagten Reaktionen der Pflanze mit einer grossen Spannbreite von Beobachtungen übereinstimmen.

In **Kapitel 2** erkunden wir die Annahmen des Modells, insbesondere wie die Modellannahmen sich zur Fitness der Pflanze verhalten. Um vernünftige Voraussagen zur Fitness zu geben wird das Modell angepasst um Konkurrenten einzubeziehen. Die Fähigkeit der Pflanze, auf Dichte und Strategien von Konkurrenten einzugehen wird beurteilt. Im Speziellen beurteilen wir ob das

Modell wirklich eine Allmendenproblematik in der Wurzelkonkurrenz voraussagt. Wir bestätigen drei Hauptannahmen des Modells: 1) Eine einjährige Pflanze muss die Wachstumsrate vor der Blüte maximieren. 2) Der Stickstoffhöhepunkt stellt eine absolute, obere Limite zur Blühzeit dar, und eine Pflanze in Konkurrenz wird zur Blüte während dem ungefähren Stickstoffhöhepunkt getrieben. 3) Eine Strategie, welche daraus besteht, alle Produktionsmittel in die Reproduktion nach dem Stickstoffhöhepunkt zu investieren, ist erfolgreicher als andere vorgeschlagene Strategien. Da eine Überallokation in die Wurzeln keine bessere Wachstumsstrategie darstellt, sagen wir ausserdem keine Allmendenproblematik voraus, wenn die Pflanze in Konkurrenz gezogen wird - dies trotz der Tatsache, dass Konkurrenz den Lebenszyklus einer Pflanze beschleunigt.

In **Kapitel 3** erkunden wir die Flexibilität einer Pflanze, welche sich gemäss den Voraussagen der Stickstoff-Höhepunkt-Modells verhält, unter Frassdruck. Wir definieren drei hauptsächliche Frasstypen: Blattfrass, Wurzelfrass, und das Stehlen von Pflanzensaft. Wir untersuchen dann die Konsequenzen der verschiedenen Arten von Frassdruck, welche einer Pflanze wiederfahren, im Hinblick auf Fitness und Blühzeit. Wir zeigen, dass die Art, durch welche eine Pflanze konsumiert wird, wichtige Fitnesskonsequenzen mit sich bringt, welche unser Modell miteinbeziehen kann. Insbesondere zeigen wir, dass das Stehlen von Pflanzensaft die Blühzeit verzögert und für den Herbivor von Nachteil sein kann, im Falle dass zuviel Pflanzensaft gestohlen wird. Eine Pflanze kann eine grosse Menge von Blatt- oder Wurzelschäden aushalten, abhängig vom Zeitpunkt des Frassdrucks. Schliesslich zeigen wir, dass die Benutzung einer Stickstoff-Höhepunkt-Regel in auftretenden Fällen von extremen Frassdruck begrenzt sein könnte.

In **Kapitel 4** wird das Modell gegen experimentelle Daten getestet. Wir testen die Fähigkeit des Modells, Wachstumstrajektorien, Blühzeit und Seneszenz für acht verschiedene Ökotypen von *Arabidopsis thaliana* vorauszusagen, welche in Wachstumskammern gezogen werden. Die Daten zeigen, dass eine geringe Anpassung des Modells nötig ist, um mit dem Wachstum von *Arabidopsis* in Einklang gebracht zu werden. Sobald aber eine solche Anpassung vorgenommen wird, kann das Pflanzenwachstum mit hoher Genauigkeit vorausgesagt werden. Mit Hilfe von Bayes' Methoden vergleichen wir die Qualität der Modellvoraussagen gegen diejenigen von verschiedenen Alternativmodellen. Wir zeigen dass eine Stickstoff-Höhepunkt-Regel für Blühzeit die Daten besser als eine Grössen- oder Altersregel voraussagt. Darüberhinaus konnten wir in Anbetracht der Unterschiede in physiologischen Parameter zwischen den Ökotypen die ökotypspezifischen Wachstumstrajektorien und Blühzeiten voraussagen.

Teile von Kapitel 1 und 2 wurden in einem Artikel vereinigt, welcher by der Zeitschrift New Phytologist eingereicht wurde mit dem Titel "Is 'Peak N' key to understanding the timinig of flowering in annual plants?", Kapitel 3 und 4 sind in Kapitelform geschrieben und werden voraussichtlich in publizierbare Artikel umgewandelt. Das Stickstoff-Höhepunkt-Modell ist absichtlich für ein besseres Verständnis in dem Methodenteil aller Kapitel aufgeführt, was zu kleinen Überlappungen zwischen den Kapitelinhalten führt.

Chapter 1:

Is ‘peak N’ key to understanding the timing of flowering in annual plants?

Camille SE Guilbaud, Neil Dalchau, Drew W Purves, Lindsay A Turnbull.

Abstract

Flowering time in annual plants has large fitness consequences and flowering cues have been the subject of much research and debate.

We introduce a new model for a nitrogen-limited annual plant in which the complete plant life-cycle can be specified using only three physiological parameters: C uptake per unit leaf mass, efficiency of N uptake per unit root mass, and target C:N ratio. The model predicts that flowering around the time when N uptake is maximised – peak N – is likely to maximise fitness and at the very least, places an upper bound on optimal flowering time.

(3) Our model predicts that peak N – and hence flowering time – has a non-linear and non-intuitive relationship with physiological parameters and N availability and we confirm some of these predictions using published results. In particular, we explain why increasing N uptake efficiency accelerates flowering, but has no effect on size at flowering; whereas N fertilisation delays flowering and increases size at flowering.

(4) We conclude that plants are likely to have evolved to use peak N as a cue for flowering, giving them flexibility under a variety of environmental conditions. We explore the potential consequences for ecology, genetics and plant breeding.

Introduction

Flowering is a key event in a plant's lifecycle and the timing of the transition to flowering has profound fitness consequences (Law 1979, Iwasa and Cohen 1989, Levy and Dean 1998). Flowering time has therefore been the focus of a great deal of research by both evolutionary and molecular biologists who have often focussed on the kinds of rules and molecular signals that plants use to optimise this transition (Stearns 1976, Weigel and Nilsson 1995, Alonso-Blanco *et al.* 1998, Koornneef *et al.* 1998, Boss *et al.* 2004, Weiner *et al.* 2009).

Molecular biologists, mostly working with the model plant *Arabidopsis thaliana*, have invested considerable effort in understanding the molecular mechanisms behind the flowering transition (Weigel and Nilsson 1995, Van Tienderen *et al.* 1996, Blázquez *et al.* 1997, Guo *et al.* 1998, Simpson and Dean 2002, Gazzani *et al.* 2003). *Arabidopsis* commonly behaves as a winter annual, meaning that it germinates in the autumn, survives the winter in the vegetative state and flowers in the spring (Roux *et al.* 2006, Satake 2010). It has therefore evolved to be sensitive to daylength cues, and if grown in the lab under long days, it will flower much sooner than if grown under short days (Sheldon *et al.* 2000, Yanovsky and Kay 2003). Under long-day conditions *Arabidopsis* plants typically flower after 25-30 days, around half way through their full lifecycle (Alonso-Blanco *et al.* 1999, Komeda 2004). The long day inductive cue is clearly important, and the biochemical mechanism that translates day length measurement into an inductive signal for flowering has been characterised (Yanovsky and Kay 2003). The master floral regulator, *FLOWERING LOCUS T* (*FT*) allows the plant to distinguish between days of long or short photoperiod by integrating photoperiodic information via the coincidence of evening-expressed *CONSTANS* protein and light. However, beyond the biochemical understanding of the

relationship between *FT* expression, light signalling and circadian regulation, relatively little is known about the signals controlling flowering time.

Despite extensive efforts to map the genetic control of flowering (Salomé *et al.* 2011), there have been few attempts to experimentally determine why long day conditions should induce flowering, and the optimal time at which to do so. For example, why do *Arabidopsis* plants in long days flower after roughly 25 days, and not after say five or 105 days? Ignoring genetics for a moment, we ask whether an ecological perspective can bring any fresh insight to this problem that might eventually lead to a better understanding of the genetic control of flowering. It has been observed that if grown under identical conditions, *Arabidopsis* flowering times are highly repeatable (Boyes *et al.* 2001, Shindo *et al.* 2007), which seems to suggest that *Arabidopsis* uses an age-rule as a cue to flower (flower on day x). But such a rule is dismissed by the fact that changing environmental conditions can lead to changes in flowering times (Wada and Takeno 2010, Zuest *et al.* 2011). The response of flowering to these changes in environmental conditions have been labelled stress-induced flowering (Wada & Takeno, 2010), although the biochemical pathways through which this occurs have yet to be elucidated. The repeatability of flowering times under identical conditions could equally suggest a size-based rule (flower at size x), because under identical conditions, size at a particular age is itself highly repeatable. But size-based flowering is also ruled out by experiments showing large differences between biomass, leaf number and flowering day, as consequence of both genetic (Salomé *et al.*, 2011) and environmental variability (Wada & Takeno, 2010).

In the meantime, there have been many theoretical attempts to understand when annual plants would be expected to flower, assuming they have evolved to maximise fitness. The first and seminal work was Cohen (1971) which showed that an abrupt transition from vegetative growth

to flowering in annual plants would be favoured by natural selection. In our opinion, this theoretical approach to understanding flowering is highly complementary to the genetic approach because the evolutionary approach addresses why plants should flower when they do, which could be the key to understanding how this is achieved.

In Cohen's model, and many that followed (e.g.: Paltridge & Denholm, 1974; King & Roughgarden, 1982; Iwasa, 2000; Kudoh *et al.*, 2002) it was assumed that season length was the overall limiting factor. Modelling studies based on this assumption considered that the plant would evolve 'knowledge' of the typical season length and therefore develop age-based rules for flowering (see discussion in Reynolds & Chen, 1996). Other theoretical studies on monocarpic perennial plants have emphasised the importance of size-based rules (Schaffer *et al.* 1982, Obeso 2002, Metcalf *et al.* 2003, 2008, Hesse *et al.* 2008). These size-based rules emerge from a trade-off between: i) flowering early at small size but with a reduced chance of dying before flowering and; ii) delaying flowering in order to grow larger but thereby increasing the chances of dying before flowering. But as pointed out above, neither age-based nor size-based rules are consistent with experimental results for many annual plants.

Here, we adopt a theoretical approach, but instead assume that terrestrial plants are likely to have evolved in an environment where nitrogen is the key limiting factor, rather than season length (Melillo *et al.* 1993, Lebauer and Treseder 2008, Vitousek and Howarth 2008). We develop a simple model of how an optimal N-limited annual plant should allocate to leaves and roots in order to maximise its instantaneous growth rate and maintain a target C:N ratio in new tissue. As explained below, our model predicts that plants should flower no later than 'peak N', the point when the nitrogen uptake rate reaches its maximum. The peak N rule is qualitatively different from age- or size-based rules because it predicts that flowering time and size at

flowering should react in a non-linear and often non-intuitive way to various environmental factors, including soil volume, soil nitrogen, CO_2 concentration, air temperature. Importantly, we find that much of the variation in the relationship between flowering time and plant biomass documented in the literature can be explained by optimal resource allocation, as opposed to stress signalling.

Methods

We: 1) introduce a new model of *Arabidopsis* growth and development; 2) show that this model predicts that *Arabidopsis* should flower close to 'peak N'; 3) explore how the plant is predicted to respond to various genetic and environmental factors and; 4) compare our model predictions with experiments documented in the literature.

Model Description: Vegetative Growth

The key assumption of the model is that there is a finite initial supply of nitrogen, N, that declines significantly during the lifecycle of the plant due to uptake. Finite N is one of several key assumptions that we return to in the discussion. We assume further that leakage of N is negligible, and that the only further supply of N during the growing season is fertilization, if applied. The total nitrogen available to the plant (N_{tot}) is the product of the nitrogen concentration at the beginning of the experiment (N_0) and the soil volume (V); hence, $N_{tot} = N_0 V$. Nitrogen is taken up by plant roots and combined with carbon, C, to make new tissues. The amount of carbon required for each unit of nitrogen is determined by the target C:N ratio (ρ) and we assume that this does not vary over the plant's lifecycle, and is the same in all plant parts; hence without fertilization, the maximum total mass that the plant can achieve is $\rho N_0 V$ (assuming

no fertilization and ignoring the initial mass of the seed). Note that here and elsewhere, for simplicity, we use 'mass' to mean mass of carbon only.

At any time, t, the rate of N uptake by roots (U_t) is:

$$U_t = \theta M_{root,t} N_t \qquad \text{(Eqn.1.1)}$$

where θ is the nitrogen uptake efficiency per unit root mass, $M_{root,t}$ is the current root mass and N is the current nitrogen concentration. If the plant was taking up sufficient carbon at time t, then all the nitrogen taken up at time t could be matched with carbon according to the C:N ratio (ρ). In this case, the plant's potential growth rate, i.e. the rate of change in its total mass, is determined by the current root mass only, hence:

$$\hat{G}_{root,t} = \rho U_t \qquad \text{(Eqn.1.2)}$$

where $\hat{G}_{root,t}$ is read as 'root limited growth rate'. Similarly, there is a leaf-limited growth rate $\hat{G}_{leaf,t}$:

$$\hat{G}_{leaf,t} = \gamma M_{leaf,t} \qquad \text{(Eqn.1.3)}$$

where γ is the carbon uptake per unit leaf mass and $M_{leaf,t}$ is the current leaf mass. Note that we assume that γ is constant throughout development. The overall growth rate of the plant is the minimum of $\hat{G}_{root,t}$ and $\hat{G}_{leaf,t}$. The strategic plant in the absence of competition should therefore optimise its allocation to leaves and roots such that $\hat{G}_{leaf,t} = \hat{G}_{root,t}$. When this equality is not satisfied, the plant invests new growth in whichever compartment (roots or leaves) limits growth,

thus restoring the co-limitation by roots and leaves (This is a commonly-used assumption, e.g.: Iwasa 2000). We assume further that the plant is able to translocate mass from leaves into roots (but such translocation is suboptimal for a plant that flowers by peak N, and therefore does not actually occur in practise: see below).

Model Description: Flowering

Following Cohen (1971), we assume that, on flowering, the plant stops allocating to leaves or roots and instead allocates all new growth to reproductive tissues. We assume that the reproductive tissues are non-photosynthetic and hence do not contribute to carbon fixation. After flowering, the growth rate of reproductive tissues will, as before, be the minimum of the root- and leaf-limited growth rates $\hat{G}_{root,t}$ and $\hat{G}_{leaf,t}$. However, after flowering begins, $\hat{G}_{root,t}$ will necessarily decline as the N concentration in the soil declines (unless there is fertilization); whereas $\hat{G}_{leaf,t}$ will remain constant. Thus the plant, which would have been optimal ($\hat{G}_{root,t} = \hat{G}_{leaf,t}$) just before flowering, will quickly become N limited ($\hat{G}_{root,t} < \hat{G}_{leaf,t}$) after flowering. Therefore, at any time t after flowering, the plant will carry a surplus leaf mass equal to $M_{leaf,t} - \frac{\rho \hat{G}_{root,t}}{\gamma}$ (i.e., the total leaf mass, minus the leaf mass that is not needed to service the N uptake from the roots). An optimal plant will translocate as much of the surplus leaf mass as possible into reproductive mass. We therefore assume that the plant instantly translocates any surplus leaf mass into reproductive mass. However, we assume that translocation of either carbon or nitrogen from roots is impossible (as is widely thought to be the case, see Aerts *et al.*, 1992; Fisher *et al.*, 2002; Schiltz *et al.*, 2005). The leaf senescence process has no effect on total plant growth (which is limited by N uptake) or N depletion, and requires no additional

parameters or assumptions in the model. A plant following the peak N model therefore grows vegetatively until it reaches peak N, then it bolts, grow reproductive mass while senescing the leaves (Figure.1.1).

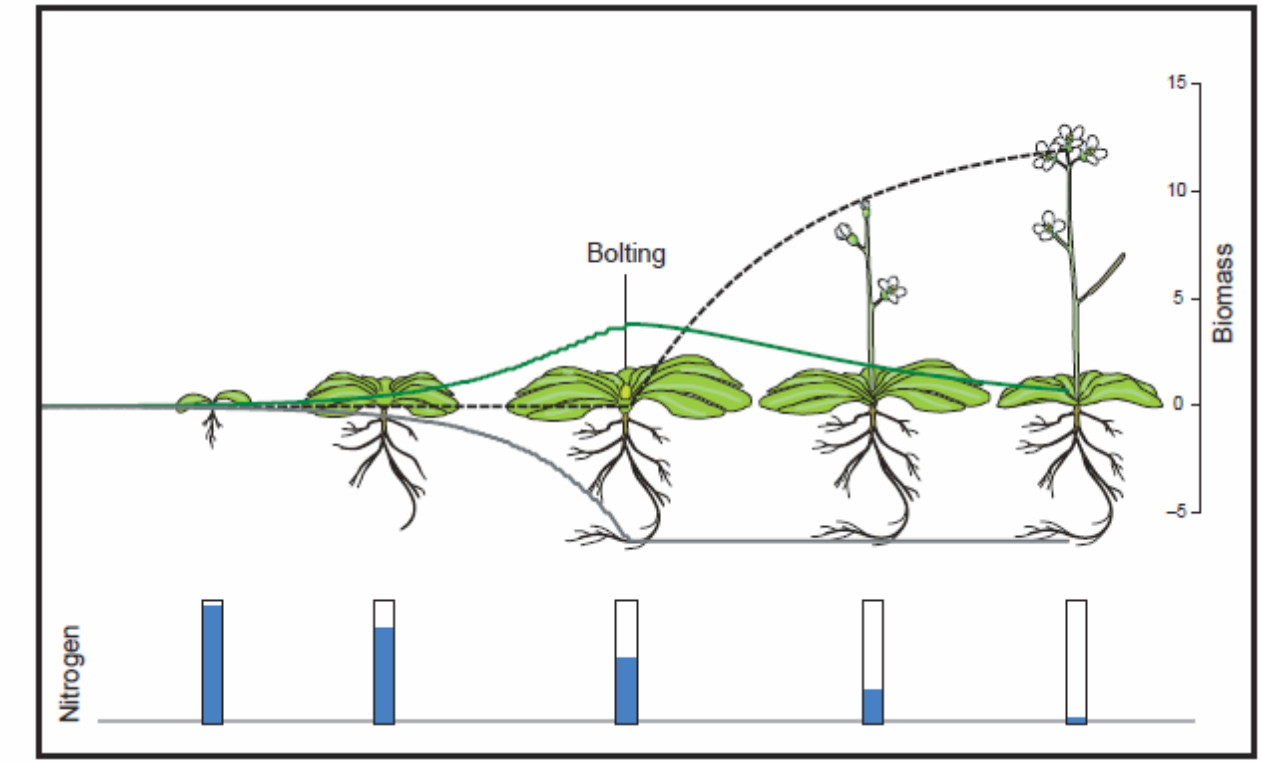

Figure.1.1. Schematic of the lifecycle of a flowering plant. Similar to the non-flowering plant, but when peak uptake is reached, the plant ceases to grow both leaves (green) and roots (brown). The plant then initiates programmed senescence of the leaves and this translocated material plus all new growth is invested directly in reproduction (dashed).

Alternatively, the plant may be able to reclaim and reallocate a high fraction of the nitrogen from the leaves, but relatively little of the carbon, as indicated by van Heerwaarden *et al.* (2003). Hence a second possibility is that following peak uptake the plant senesces only a fraction of the surplus leaf mass ($S_{leaf,t}$) and translocates only the nitrogen. The remaining leaf mass cannot be senesced as it is required to synthesise new organic carbon for this reclaimed nitrogen. Under this assumption, we can calculate the fraction (f_s) of the surplus leaf mass that can be senesced

because the amount of carbon locked up in the senesced fraction must be replaced by carbon synthesised by the non-senesced fraction, hence:

$$f_s S_{leaf,t} = \gamma(1 - f_s) S_{leaf,t} \quad \text{(Eqn.1.4)}$$

Which rearranging yields:

$$f_s = \frac{\gamma}{1+\gamma} \quad \text{(Eqn.1.5)}$$

If therefore only nitrogen can be translocated from leaves, leaf senescence takes longer and in the absence of microbial respiration we would expect to see no actual loss of leaf mass but rather a gradual yellowing of the leaves caused by the breakdown of chlorophyll for nitrogen extraction.

Model Simulations and Comparison to Experiments

We use simulations to illustrate several key predictions that arise from the model including: 1) N uptake reaches a sharp peak during development; 2) further investment in vegetative growth after peak N brings negligible benefits but locks resources in roots (from which they cannot be retrieved), implying that plants should not flower after peak N; and 3) seed mass, i.e.: initial conditions substantially affect flowering time but does not affect the reproductive output. We also use simulations to explore how several genetic and environmental factors are predicted to affect three key phenotypic measurements: age at flowering (hereafter flowering time), rosette mass at flowering (hereafter mass at flowering), and final reproductive mass, subject to the assumption that plants flower at peak N. The genetic factors considered are: 1) the target C:N

ratio (ρ); 2) photosynthetic efficiency per unit leaf mass (γ); 3) the efficiency of nitrogen uptake per unit root mass (θ). The environmental factors considered involve three different ways of altering the total nitrogen available, hence we also manipulate: 4) soil volume (V); 5) initial nitrogen concentration of the soil (N_0); and 6) continual addition of N through fertilisation (at rate φ). An increase in any of 2 – 6 above would increase the overall growth rate of the plant, but as we shall see, they have very different effects on age at flowering, size at flowering and final reproductive mass. Finally, we explore the effect of initial mass, i.e.: seed mass on flowering time and final reproductive mass.

Comparison to Experiments

We used previously published data on *Arabidopsis thaliana* to qualitatively test model predictions. We used data collected by Zuest *et al.*(2012) on 27 naturally-occurring ecotypes to test the predicted relationship between growth rates and flowering time. We also used two datasets collected on the same population of recombinant inbred lines (RILs) to test the response to increased nitrogen. In the first of these datasets, Alonso-Blanco *et al.*(1998) developed a set of 162 RILs based on a cross between the small-seeded Landsberg *erecta* (L*er*) and the large-seeded Cape Verde Islands (Cvi). They grew individuals of all lines, under typical growing conditions (fertile soil and large pots) and measured or estimated: size at flowering, flowering day and total reproductive mass (flowering day in this case is the day the first flower opens). The data is publically available (at cbsgdbase.wur.nl). In the second dataset, Paul-Victor *et al.* (2010) used 32 of these lines and also measured growth rates, size at flowering and flowering day, while in an earlier experiment using the same growing conditions they had measured the total reproductive mass (Paul-Victor and Turnbull 2009). In the experiments of Paul-Victor *et al.* (2009, 2010) plants were deliberately grown in a nitrogen-poor mixture of sand and soil in pots

of restricted diameter. Plants were notably reddened as a consequence of this growing medium (indicating nutrient stress) and senesced quickly following bolting. In addition to these data-sets, we searched the literature for experiments documenting how flowering time, mass at flowering or final reproductive mass responds to variation in the factors outlined above (initial N concentration, pot volume etc.) and compared these results to the predictions from our model.

Results

To understand how the dynamics of resource allocation might affect an optimal decision about when to flower, we simulated the growth of a single non-flowering plant (Figure.1.2). Inspection of the nitrogen dynamics (Figure.1.2a) reveals how nitrogen depletes due to plant uptake. At first N depletes slowly because N uptake is limited by plant size (Figure.1.2b), but as the root mass increases, so does the N uptake. However, N concentration in the soil continues to decline and a point is therefore reached when the increasing root mass and declining soil N concentration balance out, such that the N uptake rate reaches a maximum – we refer to this point as peak N (Figure.1.2b). Since the overall growth rate of the plant is co-limited by N, peak N also represents the time of maximum growth rate.

Before peak N, the plant maximises its growth rate through strategic investment in both leaves and roots. Because N concentration in the soil – and hence N uptake per unit root mass – declines through time, the fraction allocated to roots increases as the plant gets larger; whereas C uptake per unit leaf mass remains constant. The plant therefore becomes more ‘rooty’ in order to maintain the same ratio of N uptake to C uptake.

After peak N, N concentration in the soil begins to decline rapidly, matched by a precipitous decline in the N uptake per unit root mass (Figure.1.2b). As a result, the optimal allocation behaviour of the plant dictates that it should allocate all new growth to roots, but even so N uptake is insufficient to match C uptake. Therefore, as outlined in the model description (Methods), the plant has surplus leaf mass, which can be translocated to roots to increase the rate of N uptake at the expense of C uptake (Figure.1.2b). In this way, the plant can maintain the correct ratio of C uptake to N uptake even after peak N is reached. However, despite this reallocation, the N concentration in the soil soon approaches zero and growth stops, by which time the plant would consist entirely of roots (Figure.1.2c). Equation S.1.3 from Appendix S1.A describes the allocation process.

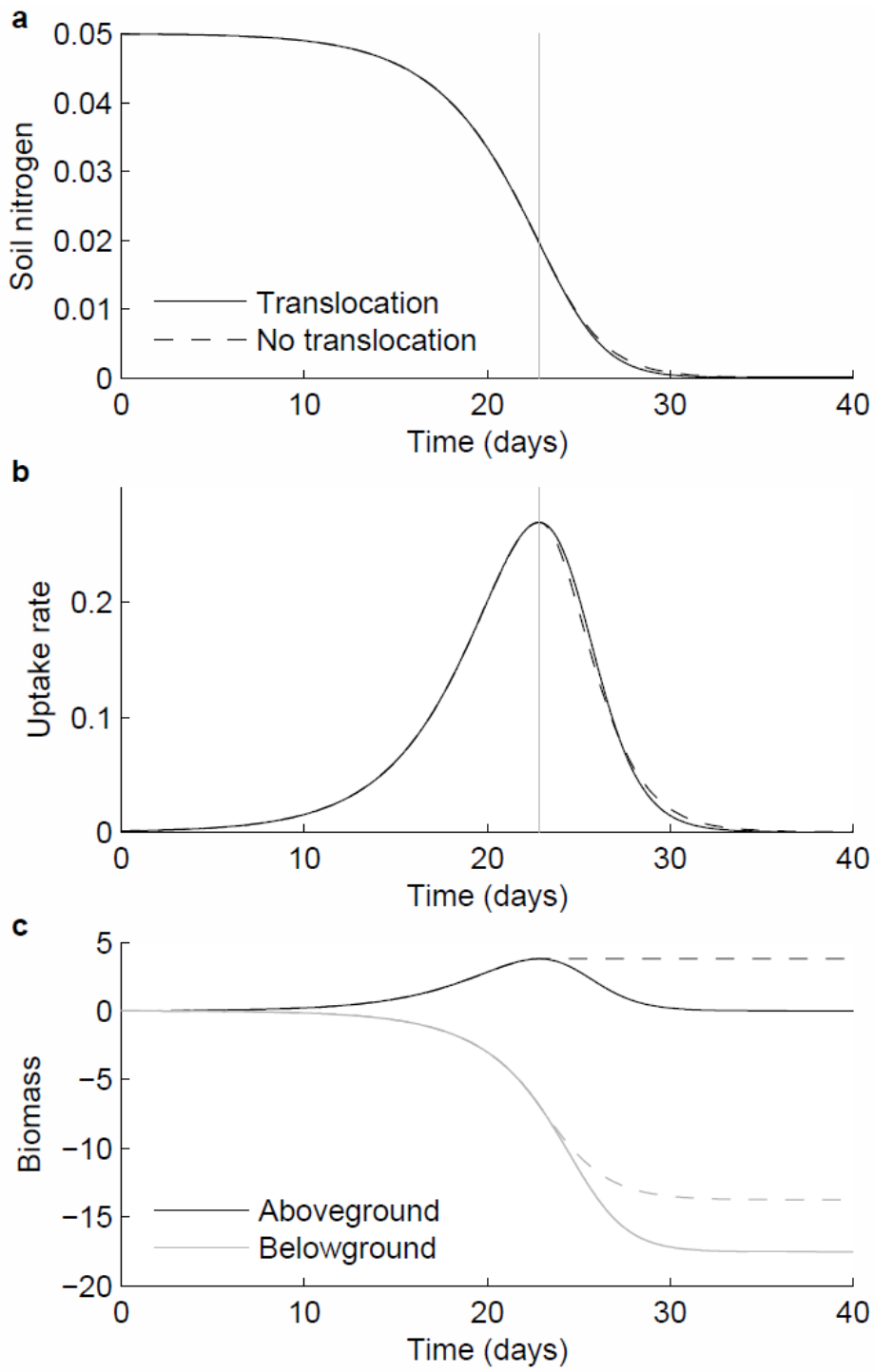

Figure.1.2 The lifecycle of a non-flowering plant. (**a**) nitrogen depletion from the soil; (**b**) the nitrogen uptake rate of the plant and (**c**) the change in biomass above (black) and belowground (grey). Peak uptake is denoted by the vertical grey line. Excess leaf mass is optionally translocated via programmed senescence into roots (solid lines), or alternatively left such that only new mass is invested in roots (dashed lines). The parameter values used in these simulations were $\rho = 7.0$, $\theta = 2.0$ cm^3 mg^{-1} d^{-1}, $\gamma = 0.5$ mg^{-2} d^{-1}, $N_0 = 0.05$ mg cm^{-3}, V = $\pi.(1.5)^2.7 \approx 49.5$ cm^3, $\varphi = 0$ mg cm^{-3} d^{-1}, seed mass = 0.02 mg.

Peak N as an upper limit to flowering

The simulation in Figure.1.2 makes it obvious why peak N places an upper limit on when to flower. Consider again peak N itself, and the period immediately following it. At peak N, the plant reaches its maximum overall growth rate, and the plant is still optimally configured for both C and N uptake ($\hat{G}_{root} = \hat{G}_{leaf}$). Immediately after peak N, if the plant continues vegetative growth, it would allocate all new growth to roots, and translocate the excess leaf mass into roots – so the root system grows very rapidly. Despite this, the N uptake rate still declines rapidly to zero. Therefore the rapid root growth that occurred after peak N achieved nothing, other than to take up the same amount of N in slightly less time; and more importantly, to lock up this nitrogen in roots, where it cannot be translocated into reproductive mass. In short, vegetative growth after peak N is pointless.

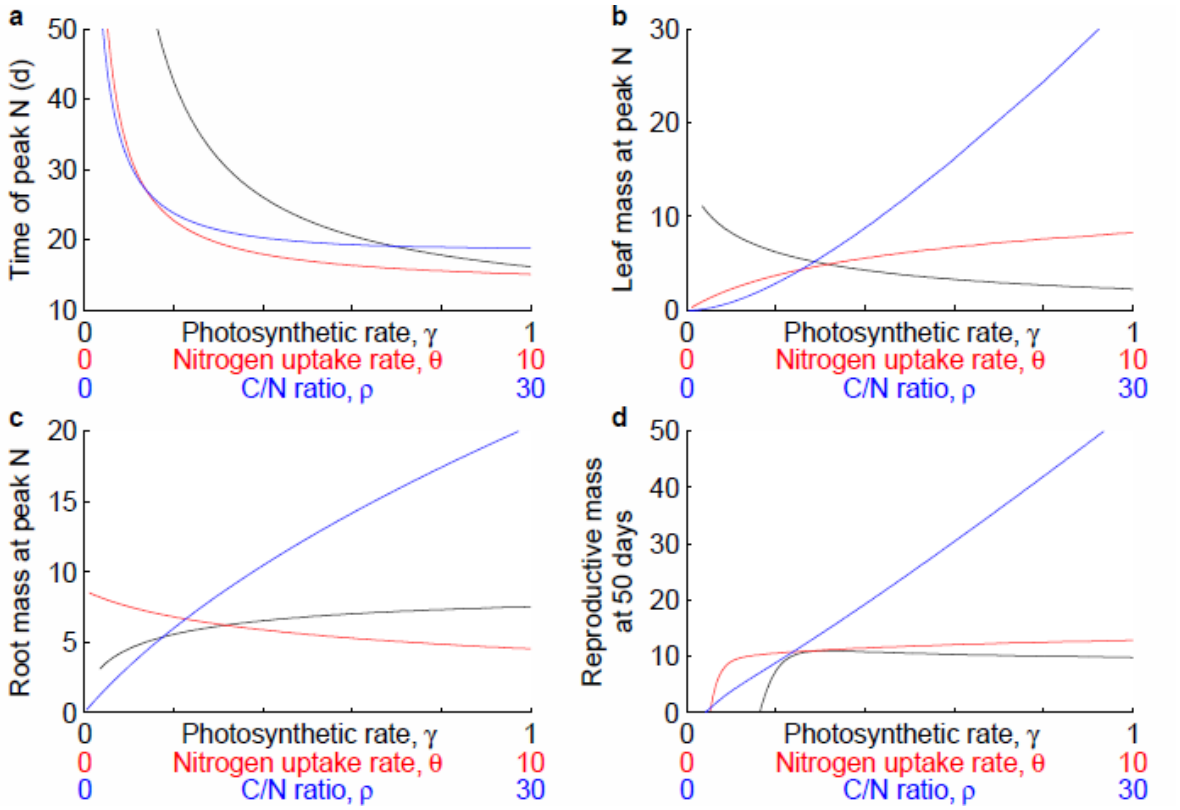

Figure.1.3. Effects of changing the leaf carbon fixation efficiency, γ, the root uptake efficiency, θ, and the target C:N ratio (ρ) on simulations of a flowering plant. The effect of changing parameter values is reported on (**a**) the time of peak nitrogen uptake, (**b**) leaf mass at peak N, (**c**) root mass at peak N, and (**d**) reproductive mass after 50 days after simulated germination. The default parameter values used in these simulations were $\rho = 7.0$, $\theta = 2.0$ cm^3 mg^{-1} d^{-1}, $\gamma = 0.5$ mg^{-2} d^{-1}, $N_0 = 0.05$ mg cm^{-3}, V = $\pi.(1.5)^2.7 \approx 49.5$ cm^3, $\varphi = 0$ mg cm^{-3} d^{-1}, seed mass = 0.02 mg.

Flowering vs carbon and nitrogen uptake efficiency

We now use simulations to explore how peak N – and by implication, optimal flowering time – depends on the physiological parameters that determine C uptake efficiency (γ) and N uptake efficiency (θ). The C uptake efficiency γ could be affected by environmental factors such as PAR, CO_2, or air temperature. Alternatively, some genetic factors could affect γ, e.g., ecotypes that invest less in defence compounds would be expected to have greater γ (Paul-Victor *et al.* 2010; Zuest *et al.* 2011). Similarly, N uptake efficiency θ could be affected by environmental

factors (e.g. soil temperature, pH, or soil texture) or genetic factors (e.g. the density of nitrogen pumps on the root hairs).

Our simulations show that increasing the rate of photosynthesis γ decreases flowering time (Figure.1.3a, black line). This occurs because, under a greater γ the plant invests less in leaves, and more in roots, thus improving N uptake, which brings forward peak N. For the same reason, increasing γ reduces the leaf mass at flowering (Figure.1.3b), but increases the root mass at flowering (Figure.1.3c). Increasing the efficiency of nitrogen uptake (θ) also decreases flowering time (Figure.1.3a, red line), because once again the faster-growing plant reaches peak N sooner. However, because in this case the roots have become more efficient, the plant now has a larger leaf mass at flowering (Figure.1.3b), and a lower root mass at flowering (Figure. 3c). Note that all effects of C and N uptake efficiency are non-linear, such that increases from low to medium efficiency have pronounced effects on mass at flowering, whereas increases from medium to high efficiency have less of an effect (Figure.1.3b,c). We also find that increasing the C:N ratio (ρ) decreases flowering time in our simulations (Figure.1.3a, blue line), leading to both increased leaf and root mass at flowering (Figure.1.3b,c). When comparing the effect of model parameters on reproductive mass, we find qualitatively distinct outcomes (Figure.1.3d). Increasing C uptake through γ leads to increasing reproductive mass until a maximum is reached at 10 mg, but further increases in γ lead to a gradual decline in reproductive mass. In slight contrast, increasing N uptake through θ leads to a similar increase to approximately 10 mg, with a subsequent moderate increase in reproductive mass. However, increasing the C:N ratio leads to a strong linear increase in reproductive mass. This is likely to be a consequence of reducing the effect of a nitrogen-limited environment by decreasing the relative quantity of N needed for new mass.

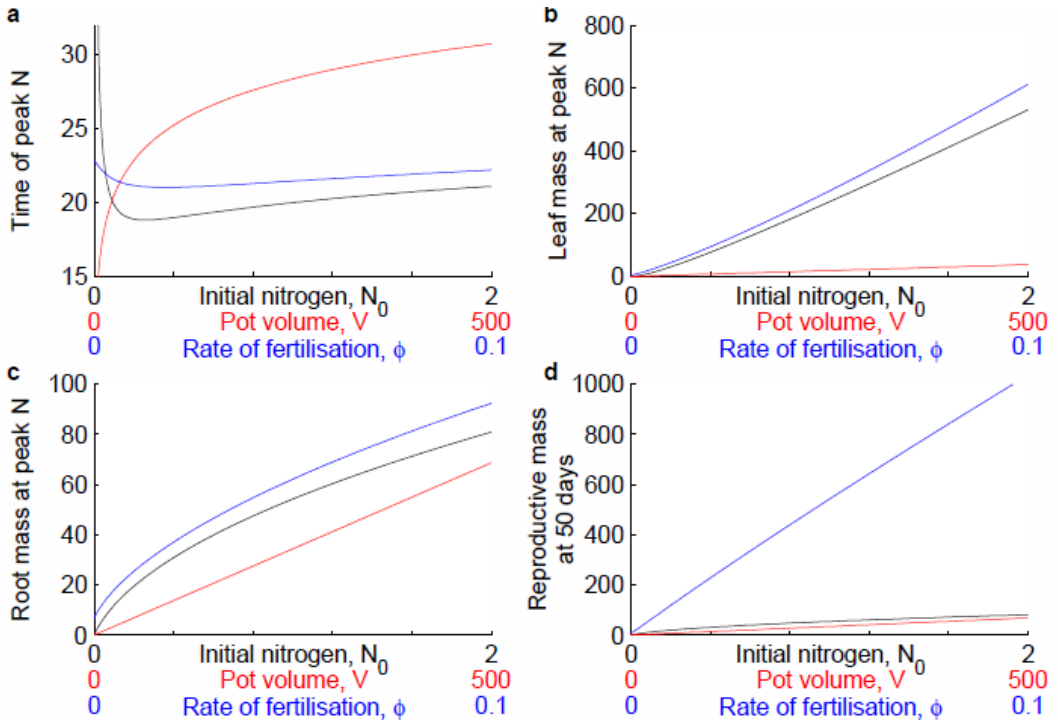

Figure.1.4. Effects of increasing total soil nitrogen on simulations of a flowering plant. The effect of increasing the initial nitrogen concentration (N_0), the pot volume (V) and the rate of fertilization (φ) on (**a**) the time of peak nitrogen uptake, (**b**) leaf mass at peak N, (**c**) root mass at peak N, and (**d**) reproductive mass after 50 days after germination. The default parameter values used in these simulations were $\rho = 7.0$, $\theta = 2.0$ cm^3 mg^{-1} d^{-1}, $\gamma = 0.5$ mg^{-2} d^{-1}, $N_0 = 0.05$ mg cm^{-3}, V = $\pi.(1.5)^2.7 \approx 49.5$ cm^3, $\varphi = 0$ mg cm^{-3} d^{-1}, seed mass = 0.02 mg.

Flowering vs nitrogen availability

There are three ways to increase N availability to the plant: increase the soil volume, increase the initial N concentration of the soil, or fertilisation throughout development. Our simulations show that increasing the soil volume causes an increase in mass at flowering in approximate proportion to the extra N provided (Figure.1.4); for example, a doubling of pot volume, approximately doubles the mass at flowering. Increasing the soil volume also makes plants flower later (Figure.1.4a). However, the relationship between flowering time and pot volume is non-linear, with flowering time increasing sharply with pot volume for small pots, then levelling off for larger pots. This non-linearity is a simple result of the near-exponential growth that occurs in the

vegetative phase of development. To a first approximation, the plant reaches peak N at a threshold size, which is proportional to pot volume (Figure.1.4a, red line). But, because growth rate increases exponentially, the time taken to reach the threshold size depends only on the logarithm of the threshold size. That is, each doubling of the threshold plant size requires only a linear increase in the number of extra days required to achieve it. An analogous result is observed in the classical logistic curve, which has a similar functional form to the growth that occurs in our model (see Eqn. S1.9 in appendix S1.B). Within the logistic, the time to the inflection point (the point at which the growth rate is greatest, analogous to peak N in our model) scales with the logarithm of the carrying capacity K (analogous to the total N availability in our model).

Increasing total nitrogen availability by increasing the initial nitrogen concentration (N_0) also increases mass at flowering but at first makes plants flower more rapidly, in contrast to increasing pot volume (Figure.1.4a, black line). Once again, flowering time versus nitrogen concentration is non-linear, showing a steep initial decrease after which it levels off and begins to increase gradually. This apparently paradoxical effect results from the fact that in this case, both the total N availability and the N concentration have been increased, whereas increasing pot volume affects total N only. The higher initial N concentration increases the initial growth rate, reducing the time to peak N; whereas increasing pot volume has no effect on the initial growth rate. Again, the effects of increasing the N concentration can be understood by analogy to the logistic. Doubling the initial nitrogen concentration is analogous to doubling both r and K which, in the logistic, reduces the time to the inflection point, because the time taken to reach the inflection point scales with the inverse of r and the logarithm of K (Eqn.S1.11 in appendix S1.B.2).

Finally, we consider increasing nitrogen availability with a fixed fertilisation rate (φ) over the growing season, analogous to watering with a nutrient solution of fixed concentration. We find that this has qualitatively similar but less pronounced non-linear effects on flowering time, leaf mass and root mass to increasing initial nitrogen (Figure.1.4a,b,c, blue line). However, reproductive mass increases an order magnitude faster than corresponding increases in N_0 (Figure.1.4d). This can be explained by the relative availability of nitrogen later in the growing season that can be achieved through fertilisation. After increasing N_0 or V, we would expect nitrogen to be relatively scarce during reproductive growth, whereas with constant nutrient additions, the progressively larger root mass continues to extract resources from increasingly fertilised soil, leading to a linear increase in reproductive mass.

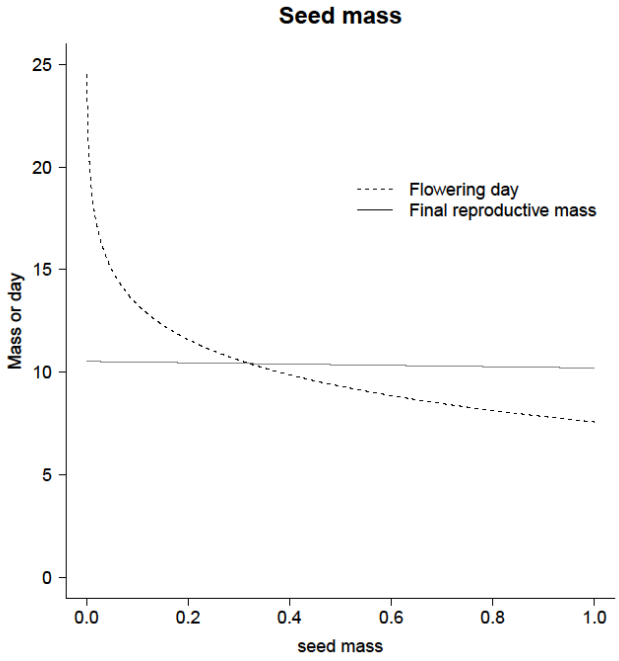

Figure.1.5. The effects of seed mass on final reproductive mass (solid line) and flowering day (dotted line) for seed masses ranging from 0.001 to 1 mg.The default parameter values used in these simulations were ρ = 7.0, θ = 2.0 cm^3 mg^{-1} d^{-1}, γ = 0.5 mg^{-2} d^{-1}, N_0 = 0.05 mg cm^{-3}, V = $\pi.(1.5)^2.7 \approx 49.5$ cm^3, φ = 0 mg cm^{-3} d^{-1}, seed mass = 0.02 mg.

Flowering vs seed mass

Variations in seed mass have no effect on the plant's final reproductive mass (Figure.1.5). However, it has an important impact on the plant's flowering day. Bigger seed mass leads to earlier flowering because of an initially bigger uptake rate. As seeds get smaller, the delay in flowering day is exponentially delayed so that most of the variation in flowering day occurs for small seed masses. For example, a difference in seed mass between 0.1 and 0.2 mg accelerates flowering by 1.7 days, while for a difference between 0.9 and 1.0 mg the acceleration in flowering is only 0.26 days (which represent approximately 6 hours).

Comparison with experimental observations in the literature

A full experimental test of the peak N model would require a raft of experiments designed specifically for this purpose, to test both the model assumptions and the model predictions. Thus, at present, our peak N model is largely theoretical. Nonetheless, we considered it interesting and worthwhile to compare key predictions arising from the peak N model, to relevant experimental results documented in the literature, where available. We find that many of the predictions of our model have been observed experimentally on at least one occasion, a result that we think should motivate more study of the peak N idea.

Flowering at the inflection point

Peak N corresponds to the time of maximum plant growth. Therefore, our model makes a simple, general prediction that, if we were to fit an appropriate sigmoid growth function to the development of an individual plant, we should find that it flowers at, or just before, the inflection point of that curve.

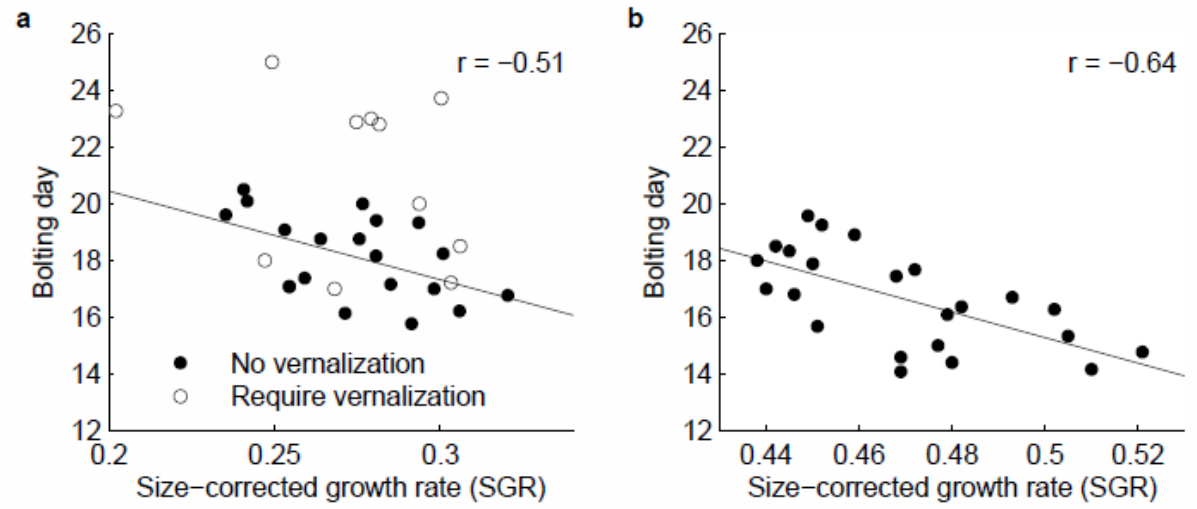

Figure.1.6. High growth rates are correlated with early flowering. (**a**) The relationship between size-corrected whole-plant growth rate (SGR) and bolting time for x recombinant inbred lines of *Arabidopsis thaliana* from the L*er* x Cvi population (data taken from Paul-Victor *et al.* 2010). Open symbols indicate lines that required vernalization for germination, while closed symbols indicate lines that did not. (**b**) The relationship between size-corrected growth rate (SGR) and bolting time for 27 natural ecotypes of *Arabidopsis thaliana* measured by Zuest *et al.* (2012).

Flowering vs efficiency of C and N uptake

Our model predicts that any genetic factor that increases the inherent efficiency of growth (either via C uptake or N uptake) will tend to decrease flowering time. The data of Zuest *et al.* (2012) and Paul-Victor *et al.* (2010) allow us to test this prediction – do lines that grow more quickly than others under identical environmental conditions, also flower earlier under those same conditions? Zuest *et al.* (2012) found a significant negative correlation between growth rates and bolting day across 27 natural ecotypes (t = –3.83, df = 21, $P < 0.001$ cor = –0.641; Figure.1.6b). The data from Paul-Victor *et al.* (2010) show a similar negative relationship between growth rate and bolting day that is marginally significant for the overall dataset (t = –1.97, df = 28, P = 0.058, r = –0.349; Figure.1.6a) and becomes stronger when lines that require vernalization are discarded (t = –2.50, df = 18, P = 0.022 r = –0.508). In addition, when the same lines grown by

Paul-Victor *et al.* (2010) were grown by Alonso-Blanco *et al.* (1999) they had: 1) an estimated rosette area on bolting day that was 18.7 times larger (t = 10.77, df = 29.053, P < 0.0001); 2) an estimated total reproductive mass that was approximately 22 times larger (t = 16.87, df = 29.094, P < 0.0001); but 3) flowered on average only 2.57 days later (t = 4.03, df = 57.97, P < 0.001) which corresponds to a 12% increase in the time to flowering. The very large increase in both size at flowering and total reproductive mass strongly suggests that plants were grown with greatly increased nutrient availability, and the increase in flowering time of a few days suggests that this is probably due to a higher pot volume (Figure.1.4a).

Our model also predicts that any environmental factor that increases C uptake will decrease flowering time, but have limited effects on mass at flowering. Although we could not find a dataset ideal for testing this idea, we did find several relevant experimental results in the literature. Higher PAR implies greater C uptake efficiency, and several papers have reported that, under long-day conditions, *Arabidopsis* flowers earlier under higher PAR (Gibeaut *et al.* 1995, Pigliucci *et al.* 1995, Tonsor and Scheiner 2007). One of these (Stratton 1998) employed a range of PAR, and found the same non-linear effect predicted by our model, with the average flowering day ranging from 40 days at 50 μmol PAR m^2 s^{-1}, to 30 days at 280 μ mol PAR m^2 s^{-1}. Whereas, over the same range of PAR, the number of rosette leaves increased only very slightly (from close to 9, to close to 10). Similarly, elevated CO_2 implies greater C uptake efficiency. Zhang & Lechowicz (1995) found that elevated CO_2 accelerated flowering, but did not affect biomass at harvest (cf. Figure.1.3d); in contrast, they found that increasing the initial N concentration had no effect on flowering date but greatly increased the biomass at harvest (cf Figure.1.4d and see below). Ward & Strain, (1997) also found a substantial reduction (approximately 10 day) in flowering day in 350 Pa vs 250 Pa CO_2; but no significant effect of

700 Pa vs 350 Pa. This is in line with the non-linear relationship between C uptake and flowering time predicted by our model (Figure.1.3a). However, in contrast to Zhang & Lechowitz (1995), Ward & Strain (1997) found that elevated CO_2 caused substantial increases in rosette mass and final reproductive mass. This may be related to the fact that Ward & Strain (1997) flooded the plants with nutrient solution, such that the conclusions of our model (based on finite initial nitrogen which then decline through development) do not apply: and indeed, under N fertilization, our model predicts that increased C uptake efficiency results in increased final reproductive mass (Figure.1.4d). More generally, Reekie *et al. al.*(1994) report earlier flowering under elevated CO_2 for four species of long-day annuals (short-day annuals showed the opposite effect); and the meta-analysis of Springer & Ward (2007) reports that 80% of annual crops exhibit accelerated flowering under elevated CO2. Springer & Ward (2007) report a mixture of effects of CO_2 on flowering time in wild annuals (approximately one quarter of species accelerated, one quarter decelerated, one quarter unaffected). Perhaps, given the results of Reekie *et al.* (1994), this results from a mixture of long- and short-day annuals being included by Springer & Ward (2007).

Discussion

By developing a model for the lifecycle of an optimal N-limited annual plant, we conclude that there is upper limit on flowering time imposed by 'peak N' – the time at which N uptake, and hence whole-plant growth rate, is highest. As a cue for flowering, peak N is only slightly more complex than a threshold age or threshold size. Yet, unlike age or size, the peak N rule predicts a raft of non-intuitive relationships between flowering time, flowering mass, and reproductive mass, versus factors such as soil volume, soil N content, the application of fertiliser, the presence

of competitors – and at least some of these predictions appear to be borne out by experiments. We therefore suggest that peak N is worthy of further consideration, and especially, further experimental testing.

Why might 'peak N' be a useful proximate signal for the best time to flower? First, and as explained above, 'peak N' marks a genuine transition for the plant, from a world where nutrients are plentiful and size is limiting, to one where nutrients are increasingly difficult to find and even massive continued investment in roots yields only a small increase in uptake. Second, peak N is highly sensitive to various environmental factors. Therefore, as a cue for flowering, the use of peak N allows a plant to plastically adjust flowering in response to the local environment. In contrast, age-based, or even size-based rules, are much less flexible – and can only adjust to the environment in evolutionary time. A third, more mechanistic reason for expecting peak N to drive flowering is that it may be relatively easy to evolve mechanisms to detect when peak N occurs. As we have shown here, by following a simple program of allocating to roots when short of N, and leaves when short of C, a plant can undergo a prolonged period of near exponential growth, during which the C:N ratio of new tissues conforms to the target ratio (ρ in our model). In contrast, once peak N is passed, nitrogen uptake declines very sharply, implying a rapid decrease in the C:N ratio of new tissue. Our analysis suggests that if plants used this drop to induce flowering, this would prevent them from flowering too late (i.e. after peak N). Intriguingly, it is already known that around half of the *Arabidopsis* transcriptome is regulated by C, N or C:N interactions (Gutiérrez *et al.* 2007), and that nitrogen signalling influences both growth and senescence (Sugiura and Tateno 2011, Trinder *et al.* 2012).

If our model is correct, it also helps to explain why pleiotropic effects in *Arabidopsis* appear to be so widespread, especially in associations between size and flowering time (Atwell *et al.* 2010,

Li *et al.* 2010) or growth (Ungerer *et al.* 2003). For example, Alonso-Blanco *et al.* (1999) found that the QTLs for seed size co-located with the QTLs for flowering time, which at first seems to make no sense. However, if plants use peak uptake as a cue for flowering, then small-seeded plants will reach peak N later and hence flower later. Thus there may appear to be linkages between genes for seed size and genes for flowering, when in fact no such flowering time genes exist. Rather, any gene affecting seed size, will naturally affect how long a plant takes to reach peak N, and therefore affect flowering time. Similarly we expect a strong negative relationship between growth rates and flowering time because faster-growing plants reach peak uptake earlier, as confirmed by the data from Paul-Victor *et al.* (2010) and Zuest *et al.* (2012).

To further illustrate this point, imagine a set of lines that differed in a set of alleles that affected just one of the parameters of our model (say, N uptake efficiency, θ). Grown under identical conditions, the lines would show correlated line-to-line differences in initial growth rate, maximum growth rate, flowering time, flowering mass, and final reproductive mass. An experimental study observing these differences would likely conclude that the N uptake genes were linked to genes for growth rate, flowering time, and so on – when in reality the only difference among the lines was N uptake itself. All lines exhibit the same true flowering behaviour, in the sense that they all employ the peak N rule. It is just that peak N is affected by differences in the uptake rate,θ. This thought experiment suggests that, to properly understand how genes affect flowering, we need to interpret the results of genetic experiments in terms of how real plants behave, rather than simply correlating various phenotypic traits against each other.

One of our key predictions – supported by the data presented here – is that plants with intrinsically higher growth rates should flower earlier, while fertiliser causes plants to flower

later. This is because fertiliser increases both the growth rate of the plant and the nitrogen pool in the environment, thus delaying peak uptake. It is well known from a wide variety of species that high levels of nitrogen fertiliser cause plants to grow vegetatively and delays flowering (Weiner 2004). We suggest that if nitrogen is supplied at too high a rate, then plants fail to receive the necessary cue for flowering. Indeed, if there is no limit on the size of the vegetative mass – for example, if the plant has a modular growth form rather than a rosette – the plant could effectively be immortalised in a non-flowering state. If the plant does flower because, for example, it has reached its maximum rosette size, we might still expect that leaf senescence would be slower compared to the plant in the declining-nitrogen environment. This is because if nitrogen can still be drawn into the plant, new carbon will still be required for continued synthesis of new tissues.

While we show that peak N uptake could be used as a useful proximate flowering cue, we do not formally show that it would maximise fitness. This would require analysis of plants grown in competition to establish the evolutionarily stable strategy (or ESS -Maynard Smith *et al.*, 1985; McNickle & Dybzinski, 2013). Even without this, to establish whether peak N is optimal for isolated plants requires more detailed information on the efficiency of translocation (Gedroc *et al.* 1996, Amthor 2000), the C:N ratio in different tissues (Gleeson 1993, Sugiura and Tateno 2011) and the relative value of seeds produced at different times (Law 1979). Ideally, it would also be useful if plants could be forced to flower at alternative times (for example by manipulating hormones that induce flowering), in order to assess the consequences of flowering time independent from other correlated factors.

Concluding remarks

In the model presented here we have focussed on the plant as an integrated whole and demonstrated why we would expect intimate connections among the major life-cycle transitions. These connections are immediately apparent once we assume a declining-nitrogen environment, but would be different in an environment with high nitrogen supply rate. Given that *Arabidopsis* has evolved in a nitrogen-limited environment, it does seems strange that this plant is so often grown under conditions that it would never normally encounter in nature. We would suggest that, to adopt a more integrated approach to studying the whole plant phenotype one should expose plants to a wider range of environmental conditions. Doing so is likely to reveal behaviours not seen in the lab, and might even reveal new rules, such as peak N flowering, which could otherwise remain hidden.

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Appendix Chapter 1

S1.A.Continuous-time model

At any time step, the plant is limited by the potential growth of either of its compartments:

$$G_{root} = \rho\theta M_{root} N \tag{S.1.1a}$$

$$G_{leaf} = \gamma M_{leaf} \tag{S.1.1b}$$

$$\text{where} \qquad N = N_0 - \frac{M_{leaf} + M_{root} - M_{seed}}{\rho V} + \phi t \tag{S.1.1c}$$

Here, the system parameters are as defined in the main text, but summarised here for completeness:

Table S.1.1: Parameters used in this study.

Parameter	Process	Default value	Unit
ρ	C:N ratio	7.0	dimensionless
θ	N uptake rate	2.0	$cm^3\ mg^{-1}\ d^{-1}$
γ	C uptake rate (photosynthesis)	0.5	$mg^{-1}\ mg^{-1}\ d^{-1}$
N_0	Initial nitrogen concentration	0.05	$mg\ cm^{-3}$
V	Pot volume	$\pi \times (1.5)^2 \times 7 \approx 49.5$	cm^3
ϕ	Rate of fertilisation	0	$mg\ cm^{-3}\ d^{-1}$
M_{seed}	Seed mass	0.02	mg

The vegetative (non-flowering) model can be simulated as a discrete-time process by increasing M_{root} by $G := \min\{G_{leaf}, G_{root}\}.\Delta t$ if $G_{root} < G_{leaf}$, or otherwise increasing M_{leaf} by $G.\Delta t$. Similarly, for a flowering plant, the flowering phase of the model can be simulated by increasing M_{flower} by $G.\Delta t$ (and optionally converting M_{root} into M_{flower} with rate parameter τ). However, depending on how we select the time interval Δt, different dynamics may be observed.

We proceed by deriving an equivalent continuous-time model, in which the plant allocates continuously to both leaves and roots during vegetative growth, such that the two growth potentials are maintained equal.[1] One interpretation of this is to consider a fraction f of the growth potential is added to the leaf mass, and the remaining fraction $1-f$ is added to the root mass. i.e.

$$\frac{dM_{leaf}}{dt} = fG \tag{S.1.2a}$$

$$\frac{dM_{root}}{dt} = (1-f)\,G \tag{S.1.2b}$$

In the case where $\phi = 0$, by assuming that leaf mass and root mass match the growth potentials at time t, it is possible to derive an algebraic expression for the fraction $f = f(t)$ that maintains equal growth potential at an infinitesimal increment in time. This is achieved by substituting

$$M_{leaf}(t+\Delta t) = M_{leaf}(t) + fG\Delta t$$
$$M_{root}(t+\Delta t) = M_{root}(t) + (1-f)G\Delta t$$

into (S.1.1), and ensuring $G_{leaf}(t+\Delta t) = G_{root}(t+\Delta t)$. With some additional algebra, we obtain

$$f = \frac{M_{leaf} - \frac{\theta}{\gamma V} M_{root}^2}{M_{leaf} + M_{root}} \tag{S.1.3}$$

Using this definition of f in (S.1.2) enables simulations to be run using numerical integrators for ordinary differential equations, for example Matlab's ode45 routine [?]. However, as it was not possible to derive an allocation function f in the case where $\phi > 0$ we used a more exotic simulation approach.

[1] This corresponds to simulating the discrete-time model with an infinitesimally small time step.

S1.A.1.Numerical integration of the model

We found that the model could also be simulated numerically without first deriving the allocation fraction f. Instead, it is possible to simultaneously allocate mass according to $\frac{dM_{tot}}{dt} = \frac{dM_{leaf}}{dt} + \frac{dM_{root}}{dt} = G$ and maintain the optimal partitioning according to $G_{leaf} = G_{root}$ by integrating the differential algebraic equations

$$\frac{dM_{leaf}}{dt} + \frac{dM_{root}}{dt} - \gamma M_{leaf} = 0 \tag{S.1.4a}$$

$$\gamma M_{leaf} - \rho\theta M_{root}\left(N_0 - \frac{M_{leaf} + M_{root} - M_{seed}}{\rho V}\right) = 0 \tag{S.1.4b}$$

Equation systems of this form, i.e. $F(t, x, \frac{dx}{dt}) = 0$, can be solved numerically using Matlab's implicit differential equation solver ode15i [?]. All simulations shown in the main text use this approach.

S1.A.2.Initial conditions

An important assumption for the continuous-time model (S.1.2) is that M_{leaf} and M_{root} are always in an optimal stoichiometry (such that $G_{leaf} = G_{root}$). This can be ensured by fixing the proportion of (eventual) leaf and root mass in the seed, and then relying on optimal allocation for subsequent times. By defining $M^0_{leaf} := M_{leaf}(0)$ and $M^0_{root} := M_{root}(0)$, we are required to satisfy the following pair of equations:

$$M_{seed} = M^0_{leaf} + M^0_{root}$$
$$\gamma M^0_{leaf} = \rho\theta M^0_{root} N_0$$

This leads to the initial conditions

$$M^0_{leaf} = \frac{\rho\theta N_0}{\rho\theta N_0 + \gamma} \cdot M_{seed} \tag{S.1.5a}$$

$$M^0_{root} = \frac{\gamma}{\rho\theta N_0 + \gamma} \cdot M_{seed} \tag{S.1.5b}$$

S1.B.Algebraic analysis

S1.B.1.Analytical solution for total mass

We found that it was possible to obtain algebraic relationships between M_{leaf}, M_{root} and t that removed the need to use numerical integration, and also to accurately characterise the allocation fraction f in the previous section. This relies of first noticing that it is possible to write down M_{leaf} and M_{root} in terms of the total mass $M_{tot} := M_{leaf} + M_{root}$ and the initial nitrogen N_0. As the growth potential from leaves and roots is always matched, we have that

$$G_{leaf} = \gamma M_{leaf} = \gamma(M_{tot} - M_{root}) = \rho\theta M_{root} N = G_{root}$$

which can be rearranged to obtain M_{root} in terms of N and M_{tot}

$$M_{root} = \frac{M_{tot}}{1 + \frac{\rho\theta N}{\gamma}}$$

Similarly, N can be written purely in terms of the system parameters and M_{tot} as

$$N = N_0 - \frac{M_{tot} - M_{seed}}{\rho V}$$

Therefore, by substituting N and M_{root} as above into an equation for the rate of change of M_{tot}, we

obtain:

$$\begin{aligned}\frac{dM_{tot}}{dt} &= \rho\theta M_{root} N \quad (= G_{leaf} = G_{root}) \\ &= \frac{\rho\theta\left(N_0 - \frac{M_{tot}-M_{seed}}{\rho V}\right)\gamma M_{tot}}{\rho\theta\left(N_0 - \frac{M_{tot}-M_{seed}}{\rho V}\right) + \gamma} \\ &= \frac{\gamma\left(M_{tot} - M_{max} - M_{seed}\right)}{M_{tot} - M_{max} - M_{seed} - \frac{\gamma V}{\theta}}\end{aligned} \tag{S.1.6}$$

where $M_{max} = \rho N_0 V$.

By the variable separation method, we obtain

$$\int \frac{M_{tot} - M_{max} - M_{seed} - \frac{\gamma V}{\theta}}{\gamma M_{tot}(M_{tot} - M_{max} - M_{seed})} dM_{tot} = \int dt$$
$$\frac{1}{\gamma}\ln M_{tot} - \frac{V}{\theta}\int \frac{dM_{tot}}{M_{tot}(M_{tot} - M_{max} - M_{seed})} = t + C$$

where C is a constant of integration.

Using partial fractions decomposition on the remaining integrand completes the general solution as

$$\frac{1}{\gamma}\ln M_{tot} + \frac{V}{\theta(M_{max} + M_{seed})}\left(\ln M_{tot} - \ln|M_{tot} - M_{seed} - M_{max}|\right) = t + C \tag{S.1.7}$$

Then using the fact that $M_{tot} = M_{seed}$ at $t = 0$, we obtain the constant of integration, and rearrange to give an inverse to the general solution of M_{tot} as a function of time.

$$t = \left(\frac{1}{\gamma} + \frac{1}{\lambda}\right)\ln\left(\frac{M_{tot}}{M_{seed}}\right) - \frac{1}{\lambda}\ln\left(\frac{M_{max} + M_{seed} - M_{tot}}{M_{max}}\right) \tag{S.1.8}$$

where $\lambda := \frac{\theta(M_{max} + M_{seed})}{V}$. We can also rearrange to provide a compact form

$$\frac{M_{tot}^{\frac{\lambda}{\gamma}+1}}{M_{max} + M_{seed} - M_{tot}} = \frac{M_{seed}^{\frac{\lambda}{\gamma}+1}}{M_{max}} e^{\lambda t} \tag{S.1.9}$$

S1.C.2. Time and mass at peak growth

Peak growth is achieved at the point of inflection, i.e. at time t_{inf} such that $M''_{tot}(t_{inf}) = 0$. Starting from equation (S.1.6) but replacing $M_{max} + M_{seed}$ with $\frac{\lambda V}{\theta}$, we have

$$M_{tot} = \frac{\gamma M_{tot}\left(M_{tot} - \frac{\lambda V}{\theta}\right)}{M_{tot} - (\lambda + \gamma)\frac{V}{\theta}}$$

Differentiating with respect to t (using the quotient rule) gives

$$\begin{aligned}M''_{tot} &= \frac{\left(M_{tot} - (\lambda+\gamma)\frac{V}{\theta}\right)\left(\gamma M'_{tot}\left(2M_{tot} - \frac{\lambda V}{\theta}\right)\right) - \gamma M_{tot}\left(M_{tot} - \frac{\lambda V}{\theta}\right)M'_{tot}}{\left(M_{tot} - (\lambda+\gamma)\frac{V}{\theta}\right)^2} \\ &= \frac{\gamma M'_{tot}\left(M_{tot}^2 - 2(\lambda+\gamma)\frac{V}{\theta}M_{tot} + \lambda(\lambda+\gamma)\frac{V^2}{\theta^2}\right)}{\left(M_{tot} - (\lambda+\gamma)\frac{V}{\theta}\right)^2}\end{aligned}$$

Equating to the numerator to zero yields inflection points when either $M'_{tot}(t) = 0$ or

$$\left(\frac{\theta}{V}M_{tot}\right)^2 - 2(\lambda+\gamma)\left(\frac{\theta}{V}M_{tot}\right) + \lambda(\lambda+\gamma) = 0$$

which has the solutions

$$M_{tot} = \frac{V}{\theta}\left(\lambda + \gamma \pm \sqrt{\gamma(\lambda+\gamma)}\right)$$
$$= M_{max} + M_{seed} + \frac{V\gamma}{\theta} \pm \frac{\sqrt{V\gamma\left(V\gamma + \theta(M_{max} + M_{seed})\right)}}{\theta} \quad \text{(S.1.10)}$$

Substituting the lower branch of this into (S.1.8) and rearranging gives an expression for the time of inflection during plant growth

$$t_{inf} = \left(\frac{1}{\lambda} + \frac{1}{\gamma}\right)\ln\left(\frac{V(\lambda+\gamma-\sqrt{\gamma(\lambda+\gamma)})}{\theta M_{seed}}\right) + \frac{1}{\lambda}\ln\left(\frac{\theta M_{max}}{V(\sqrt{\gamma(\lambda+\gamma)}-\gamma)}\right) \quad \text{(S.1.11)}$$

Chapter 2:

Exploring the assumptions behind the “peak N” model

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Abstract

The peak N model describes how an annual plant growing in a nitrogen-limited environment can optimise its lifecycle. The model includes three main assumptions: i) during the vegetative phase the plant maximises its growth rate through strategic allocation to roots and leaves; ii) the plant flowers at peak N; and finally; iii) the plant stops growing roots and senesces the leaves once flowering is initiated. We generalised the model to include competition between two or more plants sharing the same pot. By allowing different strategies to compete against each other we were then able to test the assumptions behind the model. We found that assumptions i-iii are sensible and will optimise fitness for a range of different environments. We conclude that annual plants might reasonably be expected to comply with the peak N model and that experimental investigation is now required to verify this assertion.

Introduction

Fitness, composed of individual survival and fecundity, is the measure of success in nature (Mills and Beatty 1979). Annual plants, because they die at the end of the season, tend to be selected for improved fecundity at the expense of improved survival (Grime 1977, Bonser *et al.* 2010). Ignoring survival, one therefore concludes that a successful annual plant is the plant able to produce the greatest reproductive mass. Maximizing the reproductive output over a growing season has been treated as an optimisation problem that led to the development of the first annual plant models (Cohen 1971, Paltridge and Denholm 1974). The main finding of these models is that fitness is maximised under a two-phase growth regime separated by a sharp transition from vegetative to reproductive growth. Models refining the original work from Cohen included extensions to describe perennial plants (Cohen 1976, Rees *et al.* 2006), a clearer definition of the transition time (King and Roughgarden 1982, 1983), or more aspects of a plant lifecycle (Mirmirani and Oster 1978, Iwasa *et al.* 1996, Iwasa 2000). The ability to sense when the time to flower has come is widely accepted as an important fitness trait (Stearns 1976, Simpson 2002, Metcalf 2008) and has received sustained attention for the past 20 years (Bernier *et al.* 1993, Izawa *et al.* 2000, Simpson and Dean 2002, Boss *et al.* 2004). Annual plants are modelled as growing as fast as their physiology and their environment allows them to (Coley *et al.* 1985, Wilczek *et al.* 2009, Rose *et al.* 2009) and, once flowering is initiated, they divert all new production for the growth of reproductive structures. This implies that plants should initiate flowering at different times depending on the conditions they are growing in or depending on physiological constraints (Iwasa *et al.* 1996, Stinchcombe *et al.* 2004, Nord and Lynch 2009). More recently, Kudoh *et al.* (2002) explored theoretically the intrinsic cost of delayed flowering and concluded that selection should favour early-flowering and large plants. Our model also

assumes a maximisation of growth rate before flowering and an "all-for-flower" strategy once flowering is initiated. However, it assumes that the plant should initiate flowering at, or around, peak N, when the plant nitrogen uptake is maximised. But the prediction of flowering at peak N occurs from a different mechanism because our model does not treat the plant lifecycle as a fitness optimisation problem. In this respect, our model resembles the coordination models developed originally by Thornley in 1972 (reviewed in Thornley 1998) and later refined by Chen and Reynolds (1996, 1997). Coordination models give a mechanistic explanation for a plant root-shoot allocation and growth rate maximisation but do not attempt to come up with an optimal flowering day. It is important to notice therefore that our model does not try to maximise fitness *per se*, but rather describes how a plant maximises its use of belowground nitrogen. Flowering appears from the peak N model as the moment when returns from further investment in vegetative tissues becomes negligible, rather than the moment that maximises fitness. In the previous chapter, we presented the peak N model and made specific assumptions concerning the behaviour of the plant for several components of the plant's life. First we assumed that before flowering, the plant allocated to roots and leaves in order to maximise growth rate. Second, we argued that peak N − the day the plant reached its maximum nitrogen uptake − would provide a useful signal for the initiation of flowering. Finally, we assumed that after peak N had been reached, the plant would use an "all-for-flower" strategy in which all new production is diverted to the reproductive structures. Thus, there would be no further growth of roots or leaves post-flowering. We further assumed that the leaves would be senesced according to an optimal program, as the plant has too much leaf mass once peak N has passed. These three components of an annual plant lifecycle, the allocation to roots and leaves before flowering (Fitter *et al.* 2002) , the timing of flowering (Ehrlén and Münzbergová 2009), and the production of

reproductive mass post-flowering (Farris and Lechowicz 1990), influence plant fitness. Because our model does not specifically address fitness, it appears necessary to provide at least theoretical evidence that growing according to the peak N model does indeed maximise a plant's fitness, and that following peak N is a better strategy than alternatives (i.e.: it leads to a higher fitness).

To compare the fitness of different strategies, it is essential to include competition (Wall and Begon 1985). Competition is an important driver of selection in plants (Chesson 2000, Damgaard *et al.* 2002), and the strategy that maximises the fitness of an individual growing alone, may not be the same as the strategy that maximises the fitness of an individual growing in competition (Schwinning and Weiner 1998). Competitors, for they use the same resources will influence the growth of a focal individual differently according to their size (Stoll *et al.* 2002), their physiology (Trinder *et al.* 2012), or the relative distance to the plant (Purves and Law 2002, Weiner and Damgaard 2006). Furthermore, game theoretical models of competition predict that when plants are grown together a *tragedy of the commons*, *sensu* Hardin(1968), may occur (Huston and DeAngelis 1994, O'Brien *et al.* 2007, Berger *et al.* 2008). The *tragedy of the commons* is the reduction of a collective resource through the selfish behaviour of one or a group of individual. For example, in Gersani *et al.* (2001), plants that invest more in roots gather more nutrients than their neighbours and outgrow them but decrease the "collective" fitness because nutrient uptake is a decelerating function of the root production. This behaviour is costly for the group but optimal in the evolutionary sense. Increased allocation is costly only if investment in roots can't be reclaimed later on (Aerts *et al.* 1992, Fisher *et al.* 2002, Schiltz *et al.* 2005). Distinguishing among all the components of competition is difficult but may result in agricultural benefits (Stoll *et al.* 2002).

In this chapter, we modified the peak N model to include competition. We explore: i) the effects of competition and density on the plant's lifecycle; ii) whether our pre-flowering allocation rule is optimal by comparing the fitness generated by such behaviour with the fitness of a plant that employs alternative allocation rules; iii) alternative post-flowering strategies that we compete against the peak N plant post-flowering strategy. The assumptions provided in the previous chapter generally entail the best strategy and therefore suggest that the peak N model is a useful tool for understanding environmental effects on a plant's lifecycle.

Materials and Methods

To explore the assumptions of the peak N model, we compare the fitness of a plant following the strategy described in Chapter 1 with plants following different strategies for each of the three major components of the lifecycle: the pre-flowering allocation, the timing of flowering and the post-flowering allocation. Because we model annual plants, we can measure fitness as the final reproductive mass of the plant at the end of the season. To ensure a strategy is unbeatable (it is the best strategy), we compare the fitness of a plant performing a given strategy without competition and when growing with a competitor. The modelled competitor can be a non-flowering individual with the same growing strategy as the focal plant, several non-flowering competitors with the same growing strategy as the focal plant, or a flowering individual with a different growing strategy. All simulations are performed using the discrete-time version of the model (described in Chapter 1) where plants have an original seed mass of 0.02 mg, and the following physiological parameters $\theta_i = 2$, $\rho_i = 7$, and $\gamma_i = 0.5$. The environmental conditions assumed are a pot with 30 mm diameter and an initial concentration of nitrogen of $N_0 = 0.05$.

Generalization of the model

To include competition, we adjust the way nitrogen depletes from the pot and the allocation to leaves. The amount of nitrogen in the pot at the beginning of the growing season, N_0V, decreases as it is removed by the plants. Therefore, the current nitrogen contained in the pot, N_tV, depends simply on the initial amount of nitrogen minus what has been removed by the plants, and hence:

$$N_t = N_0 - \frac{1}{V}\sum_i \frac{M_{tot,i,t} - S_i}{\rho_i} \qquad \text{(Eqn.2.1)}$$

The presence of competitors also modifies the allocation to leaves. With the addition of competitors, this allocation is different as a consequence of the change in the way the nitrogen depletes and is obtained the same way as the allocation was found in the previous chapter i.e.: by integrating the rate of allocation to leaves and roots (appendix S.2). Hence:

$$f_{leaf,i,t} = \frac{M_{leaf,i,t} - \frac{M_{root,i,t}}{\gamma_i V}\sum_{j=1}^{n}\left(\theta_j M_{root,j,t}\right)}{M_{root,i,t} + M_{leaf,i,t}} \qquad \text{(Eqn.2.2)}$$

To obtain equation 2 we considered that the plant does not actively modify its allocation in response to the presence of neighbours. Therefore, the competition experienced by the plants is simply an adjustment of the root-leaf allocation based on the now faster depleting pool of

nitrogen. Thus competition occurs indirectly belowground and we assume plants do not compete for light. Equation 2 explicitly describes the leaf allocation of a plant *i* in an environment containing *n-1* competitors. When *n = 1*, equation 2 reduces to the leaf allocation pattern described in Chapter 1 (S.1.3). To model increased density, we manipulate *n-1*, the number of competitors. For simplicity, we also assume *n-1* neighbours have the same value for all their physiological parameters than the focal plant *i*. To simulate crowding, we modelled plants growing in densities of 2,4,8,16, and 32 plants therefore the value of *n* when crowding is considered is modified according to the desired density.

Pre-flowering allocation

To test whether the leaf allocation pattern defined by equation 2 is the best allocation strategy, we define a range of pre-flowering leaf allocation strategies. The alternative allocation strategies are obtained through a modification in the balance between growth potential of the roots and growth potential of the leaves in order to create under- or over-allocation to the root compartment according to

$$\hat{G}_{root,t} = x\hat{G}_{leaf,t} \qquad \text{(Eqn.2.3)}$$

Where *x* is the relative allocation to roots compared to leaves. We allow *x* to vary from 0 to 2 in steps of 0.1. When *x = 1* the leaf allocation pattern is equal to the regular allocation pattern, when *x < 1* the plant under-allocates to roots and therefore produces more leaves. Conversely, when *x > 1* the plant over-allocates to roots. In the first instance, we assess the fitness of alternative strategies by running the model for 50 days for a plant growing alone. Then we test

the fitness of alternative strategies when the plant is growing with a non-flowering competitor. The non-flowering competitor has a regular leaf allocation ($x_j = 1$) or is using the same strategy as the focal plant ($x_j = x_i$).

Timing of flowering

To determine whether flowering at peak N brings the highest fitness, we simulate flowering of the focal plant on different days in the growing season and assess the resulting fitness. We then compare the fitness of a plant growing alone with a plant growing in competition with a non-flowering competitor. In the case of a plant growing alone, we make the plant grow under various season length to explore the effect of season length on the optimal flowering day. We vary season length from 10 days to 200 days by steps of 10 days for a total of 20 different season lengths. When we model two plants growing together, the season length was fixed at 50 days and the leaf allocation strategy of the competitor was set to the same as the focal plant.

Post-flowering allocation

Once flowering is initiated, a plant is left with several post-flowering allocation options. In Chapter 1, we assumed the plant allocates all new photosynthates to reproduction; this implied that all vegetative growth would stop and that excess leaf mass senesced and was converted into reproductive mass. An annual plant that initiates flowering can increase its reproductive mass through translocation of the leaf (we discarded root translocation), through continued photosynthesis or with both, as in Chapter 1. We called this the "all-for-flower" strategy (S1). A plant may use alternative flowering strategies. For example, to gain a competitive advantage, a plant may keep growing roots in order to increase nitrogen extraction from the environment. To compare with the "all-for-flower" strategy, we only used "pure" strategies; it means a plant's

strategy consists in investing all photosynthetic growth or all translocated mass into the compartment that corresponds to its strategy. The “photosynthetic competitor” (S2) grows reproductive mass with photosynthates and translocates the excess leaf mass into roots. A plant may not senesce its leaves to maximise photosynthesis and therefore produce reproductive mass only through continued photosynthesis. Such a strategy corresponds to a“photosynthetic flowerer” (S3) strategy. The “translocative competitor” (S4) invests all new growth in roots and produces reproductive output only via translocation. Finally, translocation may be the only way for a plant to produce reproductive mass hence the “translocative flowerer” (S5) strategy (see Table 2.1). We picked these strategies as they constitute extremes post-flowering strategies in which translocation or photosynthesis can be completely turned off and therefore display coherent alternatives to test against the extreme strategy that is the “all-for-flower” (S1) one. Finally, we assume the plants allocate pre-flowering as predicted by equation 2 and that they flower at peak N. We then model plants of different strategies growing with an “all-for-flower” plant (S1) and compare their final fitness across a density gradient from 2 to 32 plants. The winning strategy is the strategy giving consistently the highest fitness.

		S1- All-for-flower	S2- Photosynthetic competitor	S3- Photosynthetic flowerer	S4- Translocative competitor	S5- Translocative flowerer
Photosynthates diverted to	Flowers	Active	active	active	inactive	Inactive
	Roots	inactive	inactive	inactive	active	Inactive
Translocation directed to	Flowers	active	inactive	inactive	active	active
	Roots	inactive	active	inactive	inactive	inactive

Table.2.1. The different post-flowering strategies. Strategies that do not produce reproductive mass are discarded.

Results

Competition stabilises flowering around peak N

The strategy that maximises fitness for a plant growing alone is to flower at the beginning of the season and effectively be only reproductive mass (Figure.2.1a). This arises because the plant we model can't reclaim the growth invested in roots, and because the model has no time constraints. Given infinite time, a plant flowering at the beginning of the season turns all of the available nitrogen into reproductive mass and therefore has a fitness equal to $\rho N_0 V$. As we constrain the season length, the advantage of early flowering progressively disappears and the optimal timing for flowering approaches peak N (21.8 days after germination). When the growing season

becomes too short (<30 days), however, flowering earlier becomes advantageous again. The optimal flowering day increases towards peak N with shortened season length because the plant now needs to increase its growth rate to produce reproductive mass in the constrained season length. For very short season lengths, the optimal flowering day decreases again, because the plant now has not enough time to grow properly and would have no fitness if it did not flower before the end of the short season.

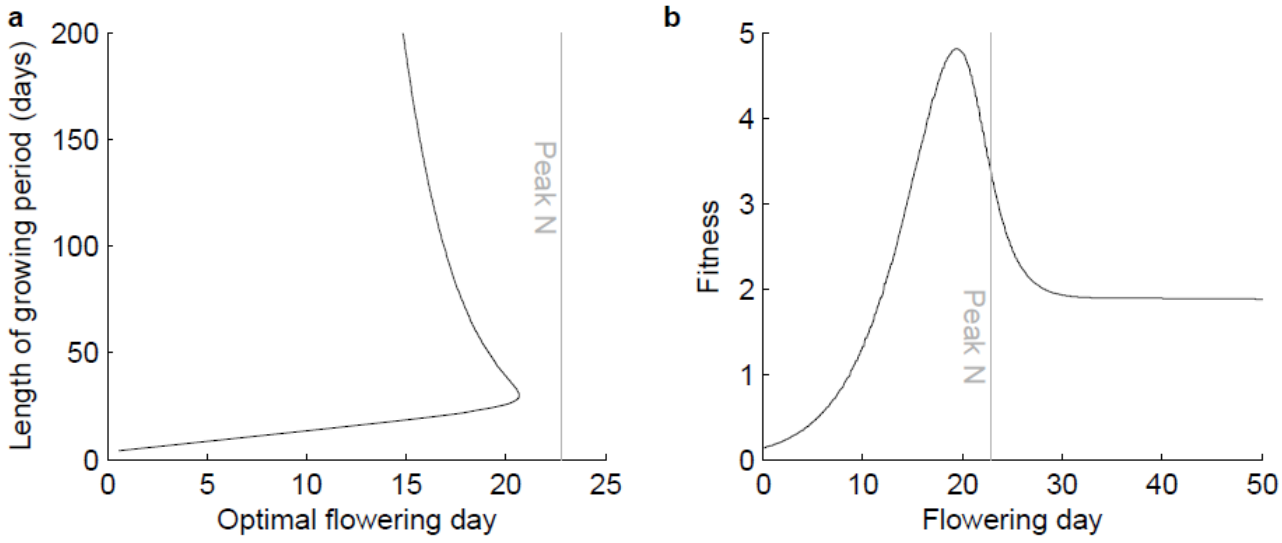

Figure.2.1. The optimal flowering day compared with peak N day. a) Simulations using a plant growing alone with different lengths of growing season (0-200 days). The final reproductive mass is very sensitive to the length of the season and therefore predicts different optimal flowering day for different season length while Peak N is insensitive to season length. b) Simulations using a plant growing with a non-flowering competitor. The final reproductive mass (fitness) varies with the day the plant initiates flowering and is maximised when the plant flowers around peak N. Parameter values: ρ = 7.0, θ = 2.0, γ = 0.5, N_0 = 0.05, seed mass = 0.02.

Grown alone, a plant is never selected to flower after peak N, and therefore the peak places an upper limit to flowering time. The addition of a non-flowering competitor in the system removes

the advantage of early flowering because a plant flowering too early now has nitrogen removed by the competitor and thus can't take up all the nitrogen present. In fact, adding a non-flowering individual displaces the optimal time to flower 2.1 days before peak N is reached with the parameters we used (Figure.2.1b). The nitrogen depletion produced by the competitor acts as a time constraint on the lifecycle of the plant.

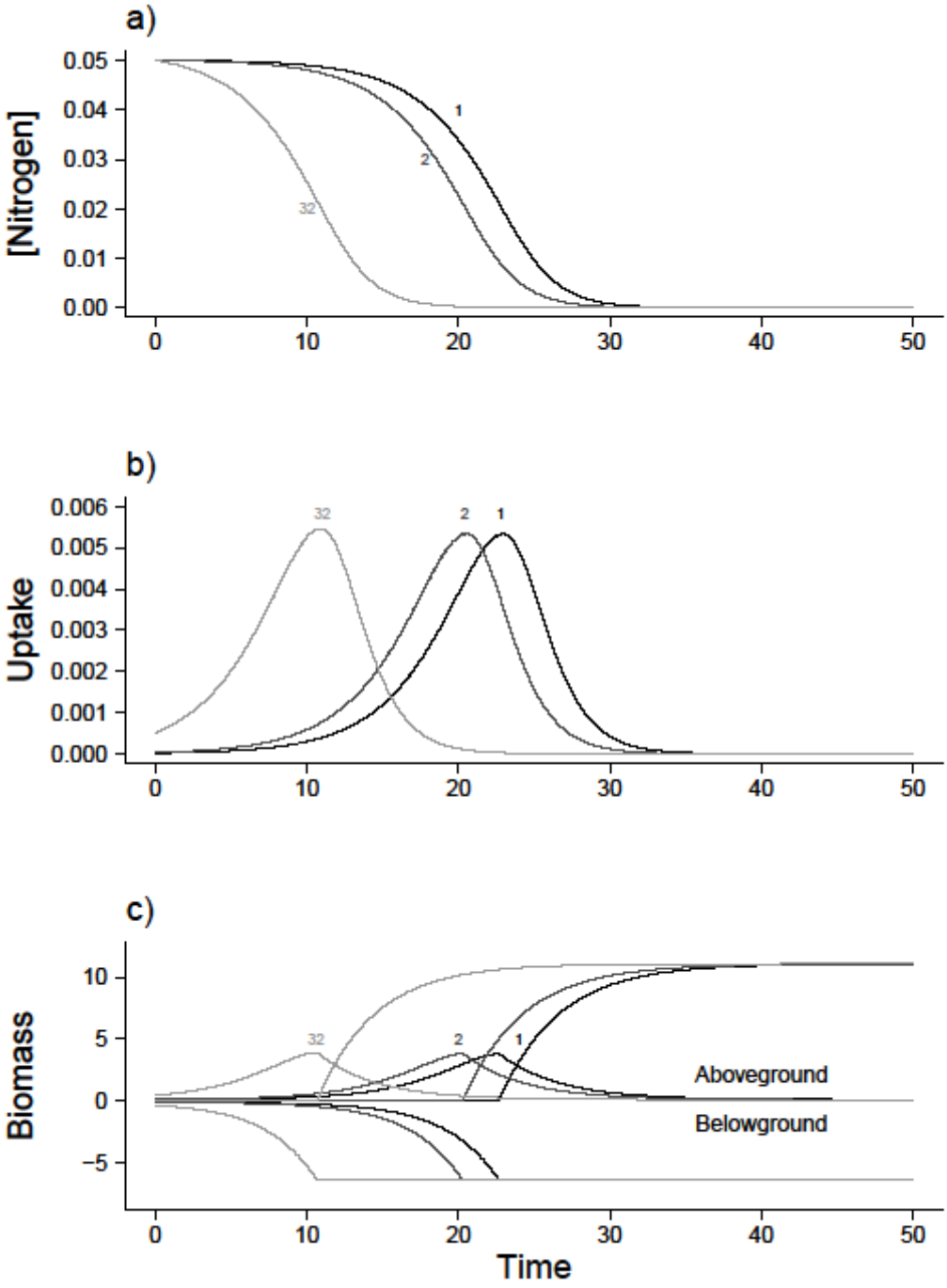

Figure.2.2. The growth of plants at various density level (1, 2, 32 plants). a) Nitrogen depletion from the soil. b) The nitrogen uptake rate. c) Change in biomass above and belowground. Parameter values: ρ = 7.0, θ = 2.0, γ = 0.5, N_0 = 0.05, seed mass = 0.02. Uptake and biomasses are multiplied by the number of individuals for comparison purposes. Nitrogen concentration is in $mg.cm^{-3}$, uptake is in $mg.cm^{-3}.day^{-1}$, biomasses are in mg.

Density induces a shift in the lifecycle timing

Adding competitors to the system has the effect of accelerating nitrogen depletion. As a result, the lifecycle of the plant is also accelerated. Nitrogen depletes faster (Figure.2.2a), peak uptake is reached earlier (Figure.2.2b), and the course of allocation is changed (Figure.2.2c). With 1 competitor (2 on Figure.2.2), peak N is reached 1 day earlier and with 31 competitors (32 on Figure.2.2) it occurs 10 days earlier. In terms of fitness, the final biomass of the focal plant represents a fraction *1/n* of the final biomass of all plants, and the total biomass by the end of the season remains constant regardless of the number of competitors, hence the presence of competitor and increased density has no other effects on the plant than a displacement of the timing of processes; there is no *tragedy of the commons* predicted from our model.

Pre-flowering overallocation does not beat 'optimal' allocation

When grown alone within a limited growing season (we used 50 days throughout), the pre-flowering allocation that leads to the highest fitness is the regular allocation described in the previous chapter, repeated in equation 2.2 (Figure.2.3a) with a final reproductive mass of 14.08 mg. Strategies of underallocation ($x < 1$) are sub-optimal because they lead to a lower root production and the limited length of the season prevents the plant from acquiring sufficient root mass to gather all the available nitrogen. Overallocation ($x > 1$) leads to a lower fitness as well because conversely, the plant has too little leaf mass to produce enough photosynthates to be as fit as a competitor with normal allocation. The regular pre-flowering allocation is optimal, it maximises the growth rate of the plant compared to alternative allocation, hence letting the plant achieve the highest fitness. With competition, the best strategy remains to allocate with $x = 1$, regardless of the opponents strategy. If the plant competitor strategy is to follow the normal leaf

allocation pattern with $x = 1$, then a strategy of root overallocation ($x > 1$) or underallocation ($x < 1$) always leads to lowered fitness (Figure.2.3b, black line), for example, $x = 1.5$, fitness = 4.01 mg compared to 7.16 mg for $x = 1$. Therefore the strategy described in our model can't be invaded. When both the focal plant and the competitor play by the same rule, the normal allocation led once again to the highest fitness (Figure.2.3b, grey line, 7.16mg) but the fitness cost of using an alternative strategy is lowered when both plants use the same strategy ($x = 1.5$, fitness = 6.68 mg). The increased final fitness is the result of the decreased growth rate which is a consequence of the inadequacy between the potential growth of the roots and the potential growth of the leaves. We do not predict that a plant can unilaterally increase its fitness through root overallocation, therefore no *tragedy of the commons* occurs. Individual fitness is maximised under the normal allocation pattern that is therefore an optimal allocation pattern.

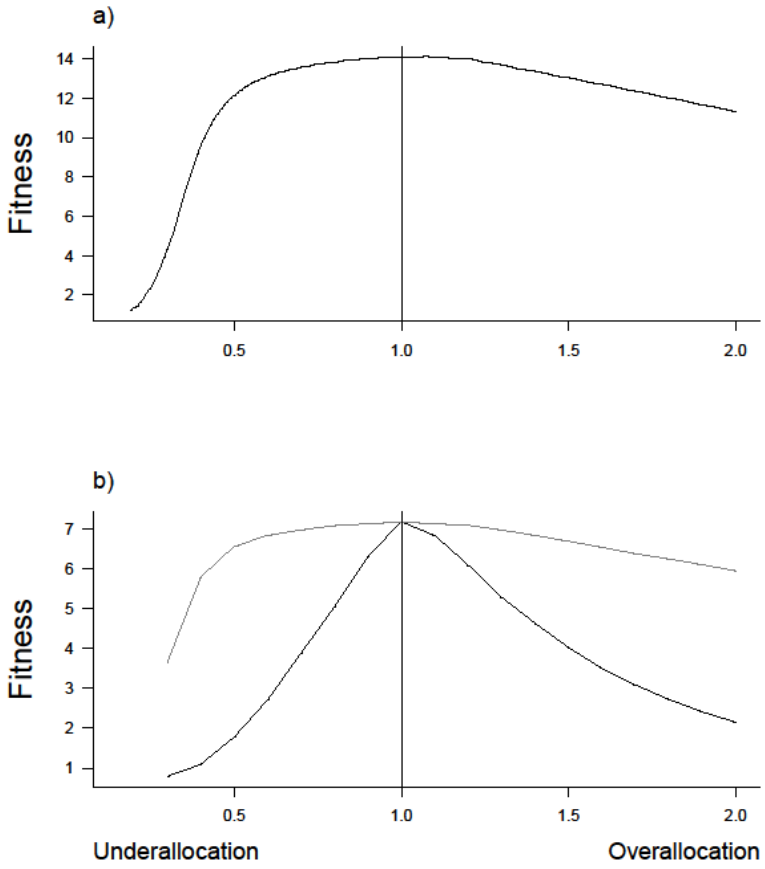

Figure.2.3. The fitness of plants using a range of root-leaf allocation strategy before flowering. Allocation varies from leaf overallocation $x \in [0; 1[$ to root overallocation $x \in]1;2]$ a) for a plant growing alone and b) for two plants growing together. The fitness outcome differs if the competitor's strategy is different from the focal plant's strategy (black line) or when both plant use the same allocation strategy (grey line). Parameter values: $\rho = 7.0$, $\theta = 2.0$, $\gamma = 0.5$, $N_0 = 0.05$, seed mass = 0.02.

The "all-for-flower" strategy is an unbeatable post-flowering strategy

When all plants are playing by the same rule before flowering and induce flowering at the same time, peak N – that is reached simultaneously by all plants – there is still the possibility to invest differently in the three plants compartments after flowering. The five strategies described above lead to different fitness only through the change in the way flowers are produced. For the set of strategies explored, the "all-for-flower" strategy (S1) displays a higher fitness than all the other strategies regardless of the density level (Figure.2.4). Translocative strategies S4 and S5 were

inferior because they did not improve fitness more than the maximum of leaf mass. The post-flowering allocation to roots from the "translocative competitor" (S4) did reduce the fitness of the "all-for-flower" plants more than the "translocative flowerer" (compare Figure.2.4c and d) but since 100% of their fitness comes from translocation while 46% (S1 vs. S4) and 28% (S1 vs. S5) for S1, an "all-for-flower" plant can always outgrow these strategies. Conversely, the "photosynthetic flowerer" strategy (S3) does not beat S1 because the "all-for-flower" strategy enhances fitness through translocation. Finally, the "photosynthetic competitor" (S2) had the second highest fitness (only 34-37% less than S1) but the extra investment in roots does not allow it to outgrow the "all-for-flower" strategy.

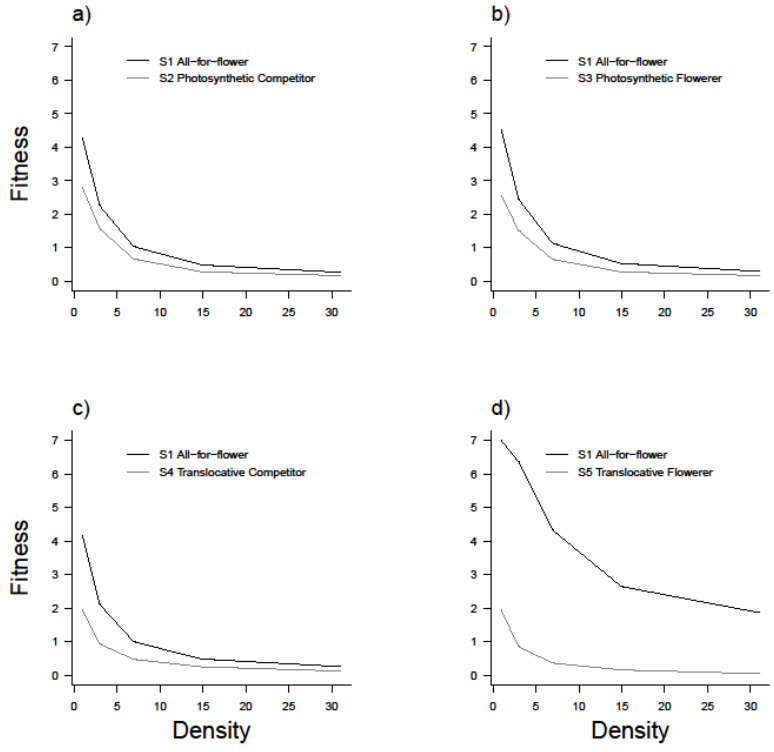

Figure.2.4. Results of competition of an individual plant fitness growing in competition with plants with different post-flowering strategies in a density gradient (1 to 32 plants). The "all-for-flower" strategy (S1, black line) is tested alone against all alternative strategies. The focal plant and competitor(s) are assumed to use the same pre-flowering allocation and to flower at peak N. Parameter values: ρ = 7.0, θ = 2.0, γ = 0.5, N_0 = 0.05, seed mass = 0.02.

Discussion

Are the assumptions behind the peak N model sensible? In-depth exploration of the assumptions revealed that i) peak N is close to the optimal flowering day when plants are grown in competition and provides an upper bound for flowering when a plant is growing alone, ii) plants following a peak N rule can adjust their lifecycle timing according to the local plant density, iii) the strategy of leaf allocation predicted by the model was protected from invasion by alternative over- or underallocation strategies and finally, iv) the best flowering strategy was "all-for-flower". All of these points make sense in the light of competition because plants are selected to race for nitrogen.

Without a race for Nitrogen, the plant flowers early

The model gives a counter-intuitive prediction for flowering day: given infinite time, a plant growing alone should flower right at the beginning of the season and have all its mass turned into seeds (Figure.2.1a). This is exactly the opposite of many predictions for flowering in the literature. Zeide, in 1978, described two evolutionary forces that act on the timing to flower: continuation of vegetative growth, which increases the reproductive potential, and the probability of death, which may annihilate the possibility for any reproduction. Zeide's first assumption that delayed flowering means more seeds leads to the prediction that, provided there is no probability of death, a plant should never flower. Our model gives such a strikingly different prediction because it considers foraging for nitrogen costly and non-refundable, contrary to other models (Cohen 1976, Iwasa 2000). The cost of growing roots is however acknowledged in competition models but the fitness definition might not rely on the production of reproductive mass (e.g.:

Gersani *et al.* 2001, use root mass as their measure of fitness). While the probability of death is important for perennial plants (Metcalf *et al.* 2003, Rees *et al.* 2006, Hesse *et al.* 2008), it is generally considered negligible for the duration of the season and increases to 1 as the season ends when annual plants are modelled (Nord *et al.* 2011),. Therefore the time constraint defined in annual plant growth models influences the optimal time to flower in optimisation models (Cohen 1971, Paltridge and Denholm 1974, Kozłowski 1992, Iwasa 2000). Getting rid of the time constraint releases the necessity for the plant to grow fast to maximise fitness and hence the variation in predicted flowering time we predict. In our model, the depletion of nitrogen sets the constraints on growth.

The race for Nitrogen makes flowering at peak N

While a plant growing alone can adjust its nitrogen uptake rate in order to minimise the cost of growing roots, it does not have this luxury when a competitor is added. By adding a non-flowering competitor, we generated a race for resource consumption. The competitor acted as a limit for nitrogen availability (Schwinning and Weiner 1998, Laird and Aarssen 2005, Damgaard and Weiner 2008) therefore to maximise fitness, a plant in competition has to adopt a flowering strategy that is balanced; if the plant flowers too early, it does not have enough root mass, if it flowers too late it has too many. The flowering strategy that maximised fitness in competition was close to peak N. This is because peak N signals the moment when further investment in vegetative tissues does not bring further fitness benefits as well as the moment of maximum growth rate. Flowering at peak N, or at least according to peak N, is an accurate strategy annual plants may use to optimise fitness.

Growing in a crowd

When we increased the number of neighbours surrounding the focal plant, we observed a decrease in the final mass of the plant, and an acceleration of the plant lifecycle with plants predicted to flower as early as 10 days after germination. This was due to the reduced amount of nitrogen the plant could gather. This result is qualitatively similar to Mirmirani and Oster (1978), who showed acceleration of flowering in competition from a fitness optimisation perspective. Acceleration of flowering time due to crowding has also been shown in *Arabidopsis thaliana* (Kozuka *et al.* 2005, Alwerdt *et al.* 2006) and was attributed to shade-avoidance syndrome (Franklin 2008). The logic behind the shade-avoidance syndrome relies on the capacity of the plant to detect and respond to changes in light quality (red/far-red ratio of light) the plant experiences. A plant following the peak N model would however, and without the capacity to perceive change in light quality respond in the same fashion. This two phenomena might be linked and further investigation could provide new insight in the way plants grow in a crowd.

Another perspective on the tragedy of the commons

Although we did not build a game theoretical model (Maynard Smith *et al.* 1985) to test the peak N model we conclude from fitness comparisons that a population of plants growing vegetatively according to the allocation equation described above cannot be invaded by a mutant overallocating to roots, or leaves (Figure.2.3b). A *tragedy of the commons*, as defined by the unilateral increase of fitness provided by overallocation for the acquisition of a common resource (Hardin 1968, Gersani *et al.* 2001, Rankin *et al.* 2007), is not predicted to occur in our model. Overallocating plants do not outcompete their neighbours because, according to our model, they just can't grow faster from higher root allocation. To maximise their growth, plants need to pay

attention to the growth potential from the roots and from the leaves. A plant overallocating to roots would be able to take up more nitrogen, but would not be able to grow leaves big enough to sustain such a nitrogen uptake. Paradoxically, allocating to roots to increase one's growth rate results in a decreased growth rate in nitrogen-limited conditions. However, we predict competition to have an effect on the allocation process and the timing of flowering (Figure.2.2c). In this respect, two points are worth mentioning. First, increased density does predict a shift in the lifecycle. At a given time *t*, a plant alone would display relatively less roots than plants grown in competition, giving the impression that competition leads to overallocation. We predict that plants grown alone could increase their fitness by flowering earlier hence having produced less roots (Figure.2.2c). In this sense, a *tragedy of the commons* occurs because competition forces a plant to follow the peak N rule when it was not without competition, and this affects fitness. This is a change of perspective: plants do not experience a *tragedy of the commons* in competition because they overallocate to roots (Gersani *et al.* 2001, O'Brien *et al.* 2007). They experience the tragedy because in competition they are in a race for nitrogen acquisition; a race a plant can only win through optimal root-leaf allocation. This race displaces the optimal time to flower at, or around, peak N and prevents a plant from the early flowering that saves them from building roots.

The race for Nitrogen implies a bang-bang strategy for flowering

Flowering is generally not considered in coordination models (Reynolds and Chen 1996, Amthor 2000, Osone and Tateno 2003, Yang and Midmore 2005). The prediction of flowering at peak N of our model is not fitness driven, hence the necessity for the present study. Fitness, here defined as final reproductive mass, is driven by the pre-flowering allocation and the timing of flowering but ultimately depends on the way reproductive mass accumulates post-flowering. Among the

five post-flowering strategies explored, the "all-for-flower" strategy was predicted to give the plant the highest fitness (Figure.2.4a-d). This is consistent with optimisation models of plant growth, particularly Cohen's (1971) and Paltridge (1974) that first predicted a "bang-bang" strategy for flowering. What our model, and theirs, say is that there is no gain from investing further in vegetative tissues post-flowering even when competition occurs. Past peak N, further investment in vegetative tissues does not bring further fitness increments and is still true when the plant has initiated flowering. The 3 strategies that diverted photosynthates to flower had higher fitness (Figure.2.4a,b). The "photosynthetic flowerer" and the "photosynthetic competitor" lacked the extra increase in fitness that comes with translocation. Investing in roots post-flowering (as in S2) revealed no fitness benefit because it occurred after the initiation of flowering. In an uncertain environment, or in an environment with potentially stochastic fertilization, a bang-bang strategy may not be optimal, as has been pointed out by several authors (Abrahamson and Gadgil 1973, King and Roughgarden 1982). In those particular cases, a photosynthetic competitor may outcompete our model plant. The translocative competitors had the lowest fitness. Although translocation is a useful process for annual plants, as shown by the "all-for-flower" strategy, it has a significantly lower fitness that "photosynthetic" strategies. It is important to point out that the surface area of the leaf is important to carry on the photosynthesis for flower and that it is not always about having more leaf mass in order to translocate more. Models such as King & Roughgarden's (1983) made the point that final yield equates to maximum leaf mass implicitly assumed a translocative strategy, which is not optimal for annual plants. However, the game may change for perennial plants where it has been shown that translocation has a great impact on fitness (Metcalf *et al.* 2008, Rose *et al.* 2009).

Concluding remarks

The race for nitrogen occurring because of competition forces a plant to maximise its growth rate pre-flowering, to flower close to peak N, and to invest all possible growth in reproductive mass once flowering is initiated to fit its growing environment. Therefore the assumptions we made for the peak N model in Chapter 1 to optimise nitrogen use are also consistent with an optimisation of fitness.

The peak N model offers a coherent story that links evolutionary necessities (optimising fitness) to ecological constraints (abiotic and biotic factors) through a decisional process that may be traceable at the genetic or molecular level. This model provides an alternative explanation to age- or size-based rules for flowering and remains consistent throughout a wide variety of environments. Because of its flexible and intricate nature, the model potentially offers new challenges for molecular biologists, physiologists, ecologists and evolutionary biologists. Experimental exploration of the robustness of the model is needed as well as further investment on the outcome predicted for other biotic factors such as herbivory and parasitism.

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Appendix Chapter 2

S.2.A. Allocation function for a plant in competition

At any time step, the focal plant is limited by the potential growth of either of its compartments:

$$G_{root,i} = \rho_i \theta_i M_{root,i} N \tag{S.2.1a}$$

$$G_{leaf_i} = \gamma_i M_{leaf,i} \tag{S.2.1b}$$

$$\text{where} \quad N = N_0 - \frac{1}{V} \sum_i \frac{M_{leaf,i} + M_{root,i} - M_{seed,i}}{\rho_i} \tag{S.2.1c}$$

$$\frac{dM_{leaf,i}}{dt} = f_i G \tag{S.2.2a}$$

$$\frac{dM_{root,i}}{dt} = (1 - f_i)\, G \tag{S.2.2b}$$

assuming that leaf mass and root mass match the growth potentials at time t, it is possible to derive an algebraic expression for the fraction $f = f(t)$ that maintains equal growth potential at an infinitesimal increment in time. This is achieved by substituting

$$M_{leaf,i}(t + \Delta t) = M_{leaf,i}(t) + f G \Delta t$$

$$M_{root,i}(t + \Delta t) = M_{root,i}(t) + (1 - f) G \Delta t$$

into (S.2.1), and ensuring $G_{leaf}(t + \Delta t) = G_{root}(t + \Delta t)$. With some additional algebra, we obtain

$$f_i = \frac{M_{leaf,i} - \frac{M_{root,i}}{\gamma_i V} \sum_{j-1}^{n} \theta_j M_{root,j}}{M_{leaf,i} + M_{root,i}} \tag{S.2.3}$$

This is the general version of the leaf allocation described in chapter 1.

Chapter 3:

Modelling the impact of herbivores using the peak N model

Camille SE Guilbaud[1], Drew W Purves[2], Lindsay A Turnbull[1-3].

Summary

In this chapter, we consider the effect of herbivores in the context of the peak N model. We modified the original model to include the impact of leaf herbivores, root herbivores and sap-suckers. Leaf and root herbivores remove a fraction of the plant's biomass in a one-time event. In contrast, sap-suckers withdraw resources from the plant during the whole growing season. We compare the predicted timing of flowering and the final reproductive mass of a plant that uses a peak N rule for flowering when subjected to the three kinds of herbivory. We contrast the performance of a plant flowering at peak N with the performance of plants flowering on any other day. We show that low-intensity herbivory delays flowering, while severe herbivory associated with sap sucking favours early flowering. Plants following the peak N rule perform well except when herbivory is severe. In such cases the plant might need to activate alternative signalling pathways. Because a plant can regrow lost vegetative tissues, leaf and root herbivory cause only a small decrease in fitness for a wide range of herbivory intensities and timing. Plants therefore have an important capacity to tolerate tissue removal by herbivores. Finally, we suggest ways in which the peak N model can be further tested using experiments with herbivores.

Introduction

Plants are the primary producers in nearly all terrestrial food webs (Melillo *et al.* 1993, Polis 1999, Purves 2013). Among them, annual plants have to deal with a wide variety of herbivores while carrying out their short lifecycle (Wada and Takeno 2010). Since they have limited time to gather resources for the next generation, herbivory can have a long-term impact on the fitness of annual plants and therefore is a strong driver of selection (Grime 1977, Chesson 2000, Elzinga *et al.* 2007, Kuang and Chesson 2008). However, herbivores differ in their feeding styles (Masters *et al.* 1993, McNickle and Dybzinski 2013), and are likely to provoke different reactions and outcomes in the plant (Huntly 1991, Agrawal *et al.* 1999, Blossey and Hunt-Joshi 2003, Poveda *et al.* 2003). For example, some authors pointed out that plant experiencing leaf herbivory may react by growing more than it would under no herbivory, a phenomenon called growth overcompensation (Belsky 1986, Kawagoe and Kudoh 2010) . Herbivory is therefore perhaps better not regarded as a single unified process (Strauss and Agrawal 1999).

Despite the wealth of data accumulated on the different forms of herbivory and their effects on plants (Stearns 1976, Strauss *et al.* 2002, Press and Phoenix 2005, Bascompte and Jordano 2007, Rankin *et al.* 2007), models exploring the effects of herbivory on individual flowering time and fitness are scarce (Coley 1980, Iwasa *et al.* 1996). Because the peak N model tracks the changes in the plant's biomass through time, it allows different kinds of herbivory and their effects on flowering time to be more thoroughly explored.

The peak N model is a theoretical model of plant growth whose main assumption is that soil nitrogen and the way it is taken up by the plant lead to a tight coordination of key stages in the life-cycle. In the model, the plant strategically invests in above- and below-ground tissues during the vegetative phase of growth, flowers when it reaches peak nitrogen uptake (peak N), and

senesces the leaves in a programmed way following flowering. Investigation of our model strongly suggests that flowering after peak N is sub-optimal. In chapter 1 and 2, we also explored how a peak N rule for flowering would shape the lifecycle of plants grown alone and in competition. In both cases, the peak N rule appeared to make sensible predictions that were supported in the literature. The peak N rule for flowering therefore seems to be a reliable and flexible cue to trigger flowering in an annual plant. However, we do not know whether a plant experiencing herbivory should also use the peak N rule.

Plants experiencing herbivory have two main lines of defence: tolerance or resistance (Karban and Myers 1989, Maschinski and Whitham 1989, Xiao *et al.* 2004). Resistance implies the production of induced or constitutive defences (Purrington 2000, Todesco *et al.* 2010), requiring a great deal of added complexity to a model (Bryant *et al.* 1983, Coley *et al.* 1985, Bazzaz *et al.* 1987). Tolerance is the ability for a plant to cope with herbivory through continued growth and, unlike resistance, does not necessarily involve the production of defensive compounds or tissues (Strauss and Agrawal 1999). In this chapter, we do not attempt to model resistance as it encompasses a wide variety of potential plant responses (Karban and Myers 1989, Herms and Mattson 1992, Endara and Coley 2011) but rather we focus on how plant tolerance to perturbation limits the negative effects of herbivory.

We explore three different common types of herbivory: one-time leaf-chewers (e.g. vertebrates, such as cows or small invertebrates such as snails but not caterpillars that typically remain on a plant for extensive periods of time), root herbivores (e.g. the larvae of certain beetles) and sap-suckers (e.g. aphids). While leaf and root herbivores are likely to act in one-time events that result in the removal of some or all of the plant parts (Belsky 1986, Poveda *et al.* 2003), sap-suckers live on the plant and continually remove potential growth (Jiang *et al.* 2003, Zuest *et al.*

2011). Because they consume different parts of the plant, herbivores with different feeding styles are likely to affect the plant in different ways. If a large percentage of a particular plant part is suddenly removed, then the plant may respond by modifying its allocation in order to replace it. In contrast, the removal of sap changes the potential growth rate of the plant, and is unlikely to affect allocation.

In this chapter, we modify the peak N model to implement the three herbivory types defined above and explore the effects of changing both the intensity and the timing of herbivory events. We explore the effects of herbivores on the growth rate, reproductive output and timing of flowering. We compare the performance of a plant following the peak N rule with a plant flowering on any other day during the growth period. This enabled us to quantify the optimality of the peak N rule when herbivores are present.

Materials and Methods

To assess the performance of a plant facing a given type of herbivory, we use a discrete-time version of the growth model developed in the first chapter. The growth model runs as follows: the plant strategically allocates to roots and leaves, maximising its growth rate until the plant flowers (Eqn.1.1-4 in chapter 1). After flowering the plant allocates all new growth to reproduction and senesces its surplus leaf mass, which is also directed into reproductive tissues. Root mass cannot be translocated under any circumstances. Because we assume in this model that the plant only has access to a fixed amount of nitrogen, the plant's nitrogen uptake increases during early vegetative growth, reaches a maximum – peak N – then decreases as nitrogen depletes. The length of the growing season is set at 50 days during which a single plant is grown in a pot of 30 mm of diameter with initial nitrogen $N_0 = 0.05$; $\theta = 2$ (nitrogen uptake efficiency);

γ = 0.5 (photosynthetic efficiency); ρ = 7 (C:N ratio) and the seed mass is 0.02 mg. In the same fashion as for chapter 2, we simulate flowering on different days and assess the resulting reproductive mass. At each step, we also assess the nitrogen uptake of the plant in order to determine the moment the plant reaches peak N. For all three types of herbivory, we compare the performance of a plant flowering at peak N with the performance of a plant flowering on the day that maximises final reproductive mass. For convenience, we refer to the plant flowering on the day that maximises final reproductive mass as "optimally-flowering". We consider how a plant could assess this optimum flowering day in the discussion.

Sap-sucking herbivores

Sap-suckers remain on the plant during the whole growing season and remove a constant fraction, α, of the plant's growth. This in effect means that sap-suckers grow in direct proportion to the plant, as the absolute mass removed by the herbivores increases through time. To model the effect of sap-sucking, we therefore modify the plant's growth rate in the following way:

$$\overline{G_t} = G_t(1 - \alpha) \qquad \text{(Eqn.3.1)}$$

Where the growth rate G_t is computed as described in chapter 1 (Eqns. 1.1 – 1.4). We varied the severity of the herbivore by changing the value of α from 0 to 1 in steps of 0.01. We also explored how the final mass of herbivores varied with the fraction of growth removed. Because G_t increases until peak N and then decreases, the herbivore growth is not exponential over the whole growing season, as is probably the case in some herbivore populations. We return to the consequences of this assumption in the discussion.

Leaf-chewing herbivores

Leaf chewers remove a fixed fraction (β) of the plant's mass in a single herbivory event occurring at time τ. We varied the fraction removed from no removal (β =0) to the removal of almost all leaves (β =0.99) in steps of 0.01: and we refer to this as the intensity of herbivory. We did not account for the case of complete herbivory (β =1), assuming that it would correspond to the plant's death. We varied the timing of herbivory τ from 0 to 50 in steps of 1 day. To model leaf removal, we ran the regular model in discrete time until τ is reached. At this moment, we modified the leaf mass in the following way:

$$M_{leaf,\tau} = (1 - \beta) M_{leaf,\tau} \quad \text{(Eqn.3.2)}$$

The model then continues to run normally until the end of the growing season. The removal of leaf mass modifies the balance of growth used in the process of allocation and hence may affect several aspects of the plant. We also need to ensure that the nitrogen removed by the herbivore is properly removed from the system, hence the nitrogen remaining after herbivory is given by:

$$N_t = N_0 \quad 1 - \frac{M_{leaf,\tau} + M_{tot,t}}{M_{max}} \quad \text{(Eqn.3.3)}$$

Root-chewing herbivores

In the same way as leaf-chewers, root herbivores remove a fraction, β, of the root mass in a one-time event. At the moment, τ, when herbivory occurs, the root mass of the plant is therefore modified as follows:

$$M_{root,\tau} = (1 - \beta) M_{root,\tau} \quad \text{(Eqn.3.4)}$$

Following this event, the model continues to follow the rules outlined in chapter 1. The nitrogen depletion is also modified to take into account the removal of some of the plant's mass by the herbivore:

$$N_t = N_0 \quad 1 - \frac{M_{root,\tau} + M_{tot,t}}{M_{max}} \qquad \text{(Eqn.3.5)}$$

Because our model coordinates growth as a balance between the growth potential of both roots and leaves, root removal may lead to quantitatively different predictions than leaf removal.

Results

For illustrative purposes, we examine first the effects of herbivory on a non-flowering individual (Figure.3.1). For these purposes only, we selected a growth removal α = 0.15 in the case of sap-sucking, and an intensity β = 0.99 on day τ = 20 for the leaf and root herbivory cases. These cases represent a removal of approximately 15% of the nitrogen initially present in the system. The rest of the parameter space in each situation is explored thoroughly below. With 15% of growth removed constantly over the growing season (Figure.3.1b), a plant experiencing sap-sucking ends up with 15% less mass (14.74 mg vs. 17.33) and is predicted to reach peak N 3.9 days later than a non-infested plant (on day 26.9 instead of day 23). In the case of leaf herbivory, we observe an immediate cessation of root growth following the herbivory event, in order to re-grow the leaves as quickly as possible. The growth of roots only starts again once the plant is back in balance. In the case of root herbivores, the opposite situation occurs: as roots are removed, leaf growth stops until the root mass has returned to the value it had before the herbivory event. The peak of nitrogen uptake is reached on day 29.4 which is 6.4 days later than a plant without herbivores. In cases of leaf and root herbivory, the final masses reached by the

plants are 14.95 mg and 14.89 mg, which correspond respectively to a 13.7% and 14% decrease in biomass (Figure.3.1c-d). For a similar withdrawal of nitrogen, the plant response is different. Sap-suckers do not dramatically modify allocation to roots and leaves but mostly act to delay peak N, and hence flowering. Leaf and root herbivores cause cessation of growth of the unaffected plant part until balance is restored, which also delays the time to reach peak N.

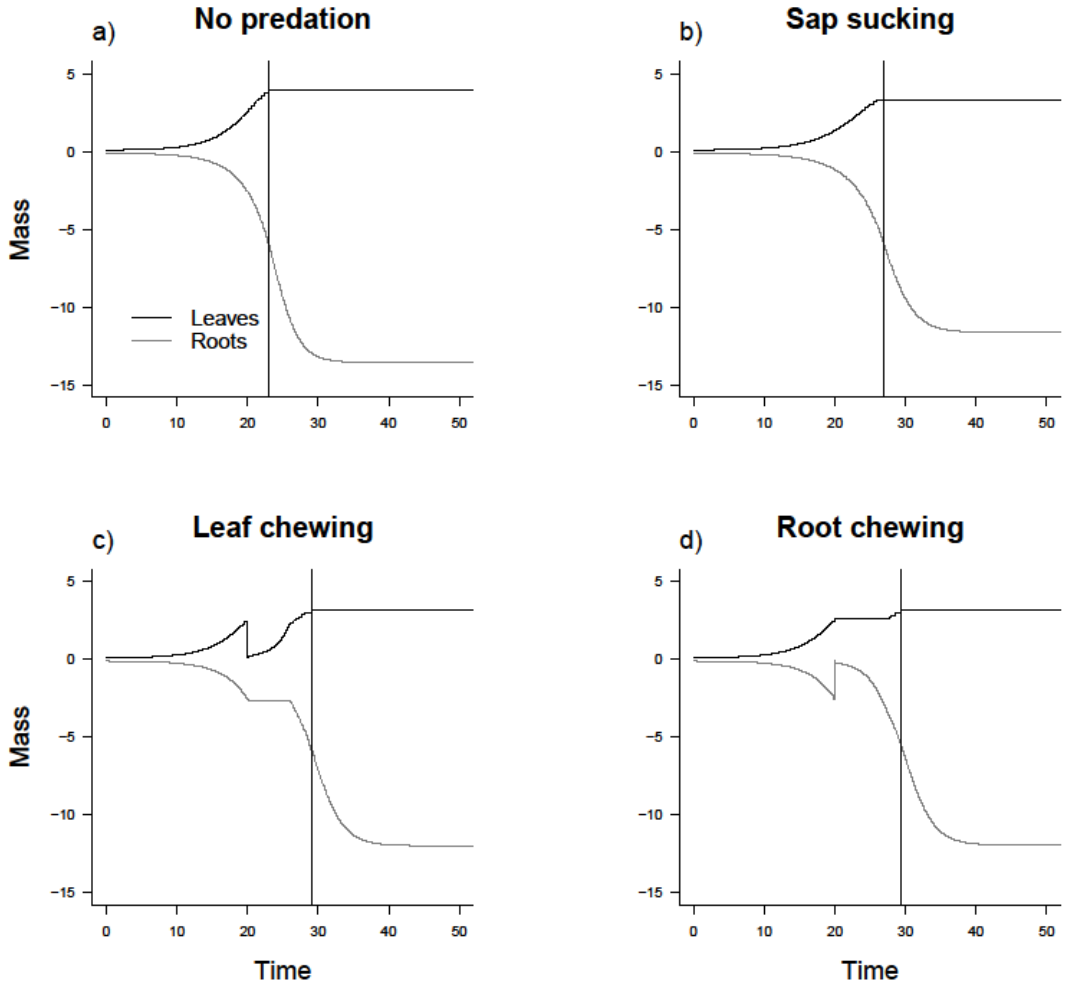

Figure.3.1. Non-flowering individuals experiencing different herbivory regimes. a) When no herbivory occurs, a plant invests in both roots and leaves until peak N is reached. At this point, no more growth of leaves occurs and the plant only grows roots. b) the continuous removal of 15% of growth reduces the plant's final mass and delays the time to reach peak N, where leaf growth stops. c) In the case of leaf herbivory, 99% of leaf mass is removed on day 20. Leaves are then regrown at the expense of roots until the plant has returned to the root-leaf balance that existed before the herbivory event. d) For root herbivory, the process is similar. Vertical lines indicate peak N. Negative mass represents belowground mass.

Sap-sucking

Sap-suckers decrease the plant growth rate which delays the time to reach peak N, hence plants using peak N as a flowering signal will flower later. Our simulations also reveal that the best day to flower – i.e. the day on which reproductive output is maximised – also declines in a similar way, until the intensity of sap-sucking passes a certain critical threshold (Figure. 3.2b). With the conditions set here, this point occurs when 61% ($\alpha > 0.61$) or more of the growth is removed (Figure.3.2b). Beyond this point, peak N is in fact never reached, hence plants using peak N as a signal to flower, never initiate flowering, and therefore produce no seeds (Figure.3.2a). When the intensity of sap-sucking is very high, the final reproductive mass is maximised by much earlier flowering (Figure.3.2b).

The fraction of growth removed throughout the season feeds the herbivore sitting on the plant. The final mass of the herbivore population therefore depends on the intensity of sap-sucking; i.e. the fraction of the plant's growth removed by the herbivore (Figure.3.1a, red line). Clearly, if the fraction of growth removed is low, the herbivore population does not perform well, so, to begin with, increasing the fraction of growth removed, increases herbivore mass. However, because the growth removed by the herbivore affects future host growth, removing too much growth also leads to low herbivore mass; hence herbivore mass is maximised at some intermediate level of growth removal. The value of sap-sucking that maximises the herbivore mass at the end of the season is $\alpha = 0.51$ for the parameter values used in our simulations. This means that a herbivore cannot increase its final mass by removing more than half of the plant's growth in our simulations.

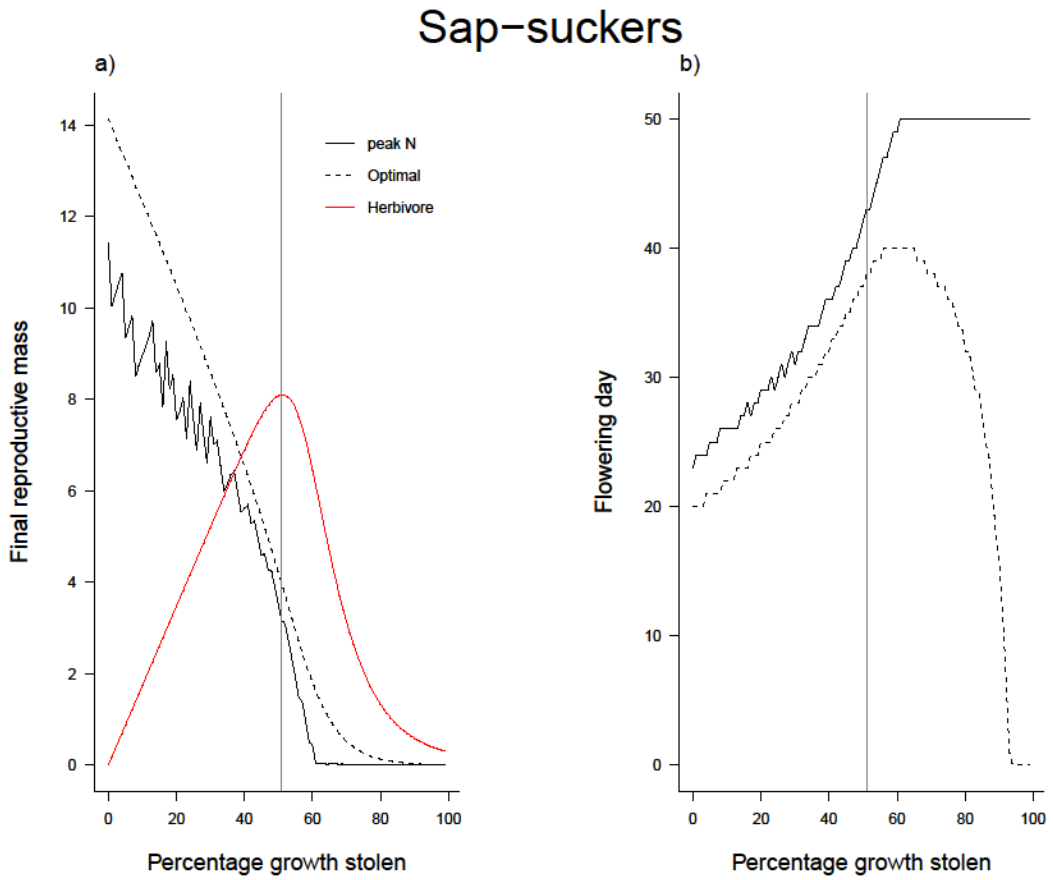

Figure.3.2. Effects of sap-sucking on a plant following the peak N rule (solid line) and a plant flowering on the day that maximises fitness (dashed line). The final reproductive mass (a) declines as the fraction of growth removed by the herbivore increases. The resulting herbivore mass (red line) is maximised at an intermediate intensity of herbivory (vertical grey line).. The optimal flowering day (b) increases until 61% of the growth is removed by the herbivore, after which, the best strategy is to flower much earlier. A plant following the peak N strategy tracks the optimal behaviour until herbivory becomes very severe..

Leaf Herbivory

Whether a plant flowers at peak N or on the day that maximises final reproductive mass, leaf herbivory reduces the plant's reproductive mass (Figure.3.3a-c) . The removal of leaf mass also slows down growth – as the plant lacks photosynthetic material – which in turn delays flowering time in both cases. However, a plant using a peak N rule for flowering is always predicted to flower later, and to accumulate less reproductive mass than a plant able to flower on the day that maximises final reproductive mass (Figure.3.3). The maximum delay associated with leaf

herbivory is 10 days in the case of a plant flowering at peak N and 12 days for a plant flowering "optimally".

If the event of herbivory occurs before flowering, the intensity of herbivory will affect the timing of flowering. The timing of herbivory does not affect the timing of flowering. As β increases, a plant is first delayed by a 1 or 2 days (Figure.3.3d) then by up to 12 days (Figure.3.3f). After flowering, a herbivore cannot affect the timing of flowering, as our model does not include the possibility of a reversion toward vegetative growth. Therefore, we cannot predict whether a real plant experiencing herbivory after it has flowered can react by reinvesting into leaves after the herbivory event. Before flowering, the intensity of herbivory is more important than the timing of herbivory. Flowering produces a breakpoint in the prediction of the effects of the timing of herbivory on flowering time because herbivory event after flowering has occurred cannot affect the timing of flowering (Figure.3.3d-f).

In contrast, the timing of herbivory has an important effect on the final reproductive mass. Particularly, a plant consumed around the predicted day of flowering has a much lower reproductive mass than a plant being consumed earlier or later in the season (Figure.3.3a-c). The main cause of this decrease in final reproductive mass is due to the variation of leaf mass through time. A plant that does not experience herbivory will grow leaves at a near-exponential rate until it reaches peak N, and will then senesce its leaves rapidly. As a consequence, the absolute loss of leaf mass is maximised when herbivory takes place around the time the plant flowers which in turn causes a greater loss in final reproductive mass. The loss of leaf mass causes a decrease in the photosynthetic capacity of the plant and in the quantity of tissues that can be translocated towards reproductive mass. In both cases, this affects the final reproductive mass. Early in the season, herbivory has little effect on the plant's final reproductive mass because the leaf mass

itself is small and because the plant can regrow leaf tissues in order to maintain its photosynthetic rate (τ =8, β =0.9, reproductive mass is 95.43% of non-consumed plant). Late herbivory has little effect on the reproductive mass as well because the mass of leaves is smaller past flowering due to senescence (τ =44, β =0.99, reproductive mass is 94.93% of non-consumed plant).

Final reproductive mass against timing of herbivory has the general shape of a V, with herbivory events occurring around the time of flowering having bigger impact on the plant reproductive mass than early or late herbivory events. This is a consequence of the nature of an annual plant lifecycle which consists in two phases. During the vegetative phase, a plant gathers resources that will later be used to build reproductive structures. When a plant is consumed at the time flowering is initiated, the herbivore nullifies the plant investment in the vegetative structure whose goal is to build reproductive structures. Another consequence of this plant lifecycle is that across intensity of herbivory the percentage of material removed early or late in the season have a low impact on the final reproductive mass.

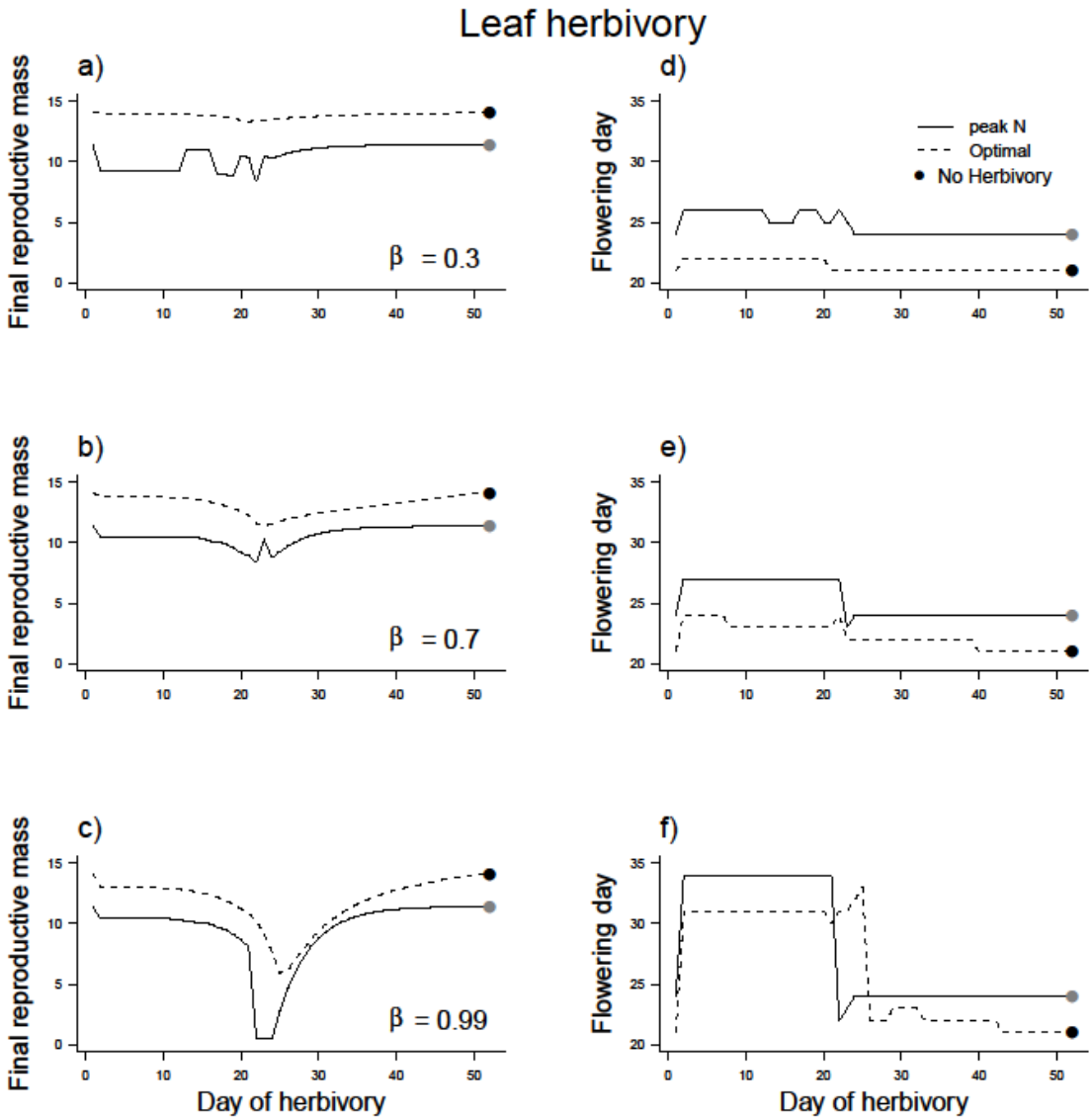

Figure.3.3. Effect of leaf herbivory on the final reproductive mass (a-c) and flowering time (d-f) of a plant initiating flowering at peak N (solid line) and a plant initiating flowering at the time that maximises reproductive output (an "optimal" plant, dashed line). Three intensities of herbivory are shown (β=0.3, β=0.7, β=0.99). In each case herbivory represents a one-time event that takes place on a single given day (day of herbivory). Flowering day and the day of herbivory are simulated using steps of one day, causing granularity. Black and grey dots represent the value of final reproductive mass and flowering time of a plant experiencing no herbivory.

Root Herbivory

A root herbivore, in a similar way to a leaf herbivore, also causes a delay in flowering and a decrease in final reproductive mass. The maximum delay in flowering following root herbivory is 9 days for the plant flowering at peak N and 15 days for an "optimal" plant. Thus, root

herbivory has potentially a greater effect on flowering time than leaf herbivory. The increased delay in flowering time after a root-herbivory event occurs because the removal of root mass decreases the plant nitrogen uptake and therefore delays peak N whereas leaf herbivory modifies the plant growth rate but does not directly affect nitrogen uptake. The maximum fitness loss due to root herbivory is 42.6% for an "optimal" plant and 66.3% for a plant following peak N. This is in both cases a lower loss compared to the maximum fitness loss from leaf herbivory.

Root herbivory has a lower impact on the plant final reproductive mass than a leaf-herbivore because the nitrogen uptake depends on both root mass and the amount of nitrogen present in the soil and because root mass is not translocated into reproductive mass. Because root mass cannot be translocated, the loss of reproductive mass consequent to root herbivory depends only on the decreased nitrogen uptake. Finally, because nitrogen uptake is bound to decrease because of soil nitrogen depletion, root herbivory, especially after the plant has flowered, causes less loss of final reproductive mass.

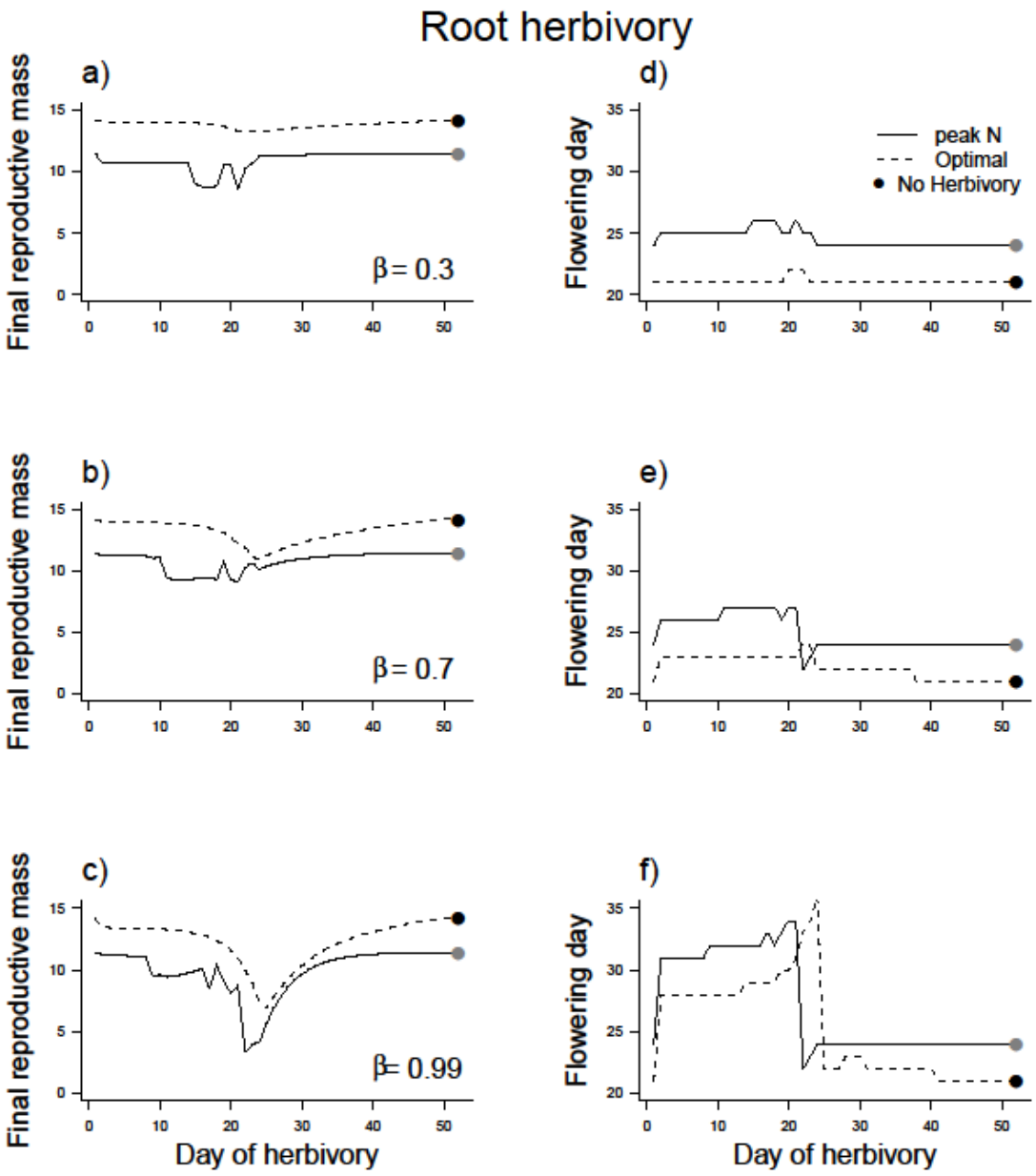

Figure.3.4. Effect of root herbivory on the final reproductive mass (a-c) and flowering time (d-f) of a plant initiating flowering at peak N (solid line) and a plant initiating flowering at the time that maximises reproductive output (an "optimal" plant, dashed line). Three intensities of herbivory are shown (β=0.3, β=0.7, β=0.99) for an event of herbivory that may occur any time in the growing season (Day of herbivory). Plants not affected by herbivory (grey and black dots) have higher final reproductive mass and flower earlier.

Comparison of effects

Tissue removal by leaf or root herbivores caused a decrease in final reproductive mass and the best response – i.e. the response that maximises the reproductive output – is always to delay the initiation of flowering. In contrast, sap-sucking would favour earlier flowering when the fraction

of growth removed is very high. Although the plant initiating flowering at an "optimal" time accumulates more reproductive mass than a plant using a peak N rule for flowering, there is a high correlation between the two flowering patterns. Along the gradient of sap-sucking, the reproductive mass produced by a plant flowering at peak N correlates higly with the reproductive mass produced by the plant flowering at the "optimal" time (r = 0.991). In the case of root and leaf herbivory, the correlation between the two plants tested is also high (r = 0.704 and r = 0.735 respectively). This suggests that peak N provides a useful guide to the plant that correctly predicts the direction of the optimal response. However, ideally, the plant would flower a short time before peak N is reached.

Discussion

The peak N model was primarily designed as a plant growth model to predict flowering time in nitrogen-limited conditions. It can, however, be used to predict the effects of herbivores that remove plant parts or who suck the sap of the plant and hence reduce it's growth rate. Our model suggests that plants can tolerate a high degree of leaf and root herbivory provided it does not occur around the time the plants flower, whereas sap-sucking may disrupt the plant lifecycle to such an extent that the plant does not flower at all. In this regard, our model confirms Strauss and Agrawal's (1999) insight that the way a plant is fed upon has a large impact on life-history traits and hence herbivory should not be considered as a single trait. Furthermore, the predictions provided in this chapter call for a set of experiments that would be straightforward to set up to test the model.

Flowering on peak N day vs. the "optimal" flowering day

Flowering according to the peak N rule is highly correlated with the "optimal" schedule across all herbivory types. The only major divergence occurred for the high-severity sap-stealing regime (with a growth removal $\alpha > 0.61$). We therefore conclude that being sensitive to nitrogen uptake rates could lead to an optimal flowering response in the face of herbivory. However, flowering exactly "at" peak N would not be optimal. We are therefore faced with the rather difficult problem that, as in other chapters, an optimal plant would flower slightly before peak N. Hence, plants that could monitor changes in nitrogen uptake rate, and accurately predict peak N, would be favoured by natural selection. However mechanistically, we do not know how this would be achieved. However, in principle it is perhaps easier to evolve than a set of individual rules for each particular environment (Reynolds and Chen 1996). The only regime from which the peak N rule deviated drastically from optimal was under the severest sap-stealing regimes. However, in this case there could be an over-ride based on a stress signal, but this would require further testing.

Sap-sucking may break the peak N rule for flowering

The theft of sap constitutes a definite and continuous loss for the plant. Our model predicts growth removal can delay, accelerate or even prevent a plant from flowering depending on the plant modelled behaviour (Figure.3.2). In this regard, theft of sap from sap-sucking insects, or a parasite is predicted to have a set of easily testable effects. In Zuest *et al.* (2011), aphid loads led to a constant growth removal α of 0.03 with a slight delay in flowering time while parasite load in Pagán *et al.* (2008) as well as in Peters (1999) showed acceleration in flowering time in one case and delay in the other case. It is possible to draw a parallel between growth removal from sap-sucking insects and the effect of parasitic plants on plant growth. For example, repression of

flowering is sometimes associated with parasitism (Leisner and Howell 1992, Jiang *et al.* 2003, Press and Phoenix 2005). Hautier *et al.* (2010) studied the parasitic plant *Rhinanthus* and modelled the effect of parasite intensity (fraction of growth removed) on the performance of both host and parasite. As here, their model predicts that the combined mass of parasite and plant is bound to be lower than the plant grown alone and that intermediate levels of growth removal provide the highest biomass outcomes for the parasite. In addition to these predictions, our model provides a quantitative measure of flowering change and reproductive output for a given level of herbivory.

We modelled sap-sucking as a function of the plant's growth. This assumption implies the herbivore on the plant can grow exponentially at the beginning of the season but it could not keep doing so during the whole season without killing its host. However, population of sap-suckers may display a lifecycle that involves an exponential growth leading to the death of the host (Dixon 1977) and our predictions may therefore minimise the effect of sap-sucking in natural settings. An experiment to test the effect of growth removal could be set up in the following way: An annual plant would be exposed to a gradient of sap-sucking loads (a range of α) over its lifecycle as flowering time and final mass are recorded. Provided the plant physiological parameters (γ, θ, and ρ for the peak N model) are estimated from the control treatment, it is possible to estimate precisely if the model gives quantitatively and qualitatively relevant predictions. Moreover, such an experiment would provide a way to confirm the existence of a mechanism that would trigger flowering when a plant experiences high herbivory rates.

Tolerance to leaf herbivory is effective

In contrast to sap sucking, dynamic optimisation of growth implemented in the peak N model predicts that a plant can mitigate the effects of herbivory. Except for herbivory occurring around the time of flowering that has the biggest effect on reproductive outcomes. The ability of plants to tolerate herbivory is well-documented (Walters and Reich 1996, Agrawal *et al.* 1999, Weinig *et al.* 2003) and therefore supports this prediction from the model. An interesting result from the exploration of herbivory is that the timing of herbivory has a crucial impact on the plant's reproductive outcome (Figure.3.3 & 3.4). Our model suggests that being consumed around the time of flowering leads to a much greater decrease in flowering time compared to any other time in the growing season. In Akiyama and Ågren (2012), plant leaves are removed at different time during the plant's lifetime. They show that just before flowering the cost of leaf removal is higher than after flowering (60% seed mass loss vs 22%), suggesting our model is at least qualitatively meaningful. Although, it is generally admitted that herbivory decreases plant fitness (Maschinski and Whitham 1989, Agrawal 1998, Adler *et al.* 2001, Tiffin 2002, Arany *et al.* 2005, Barto and Cipollini 2005, Kotowska *et al.* 2010), it seems no consensus exists concerning the flowering time. In particular, Kawagoe and Kudoh (2010) found that *Arabidopsis halleri* flowered earlier under herbivory regimes. But a delay or no effect has also been observed (Agrawal 1998, Bidart-Bouzat 2004). Finally, our model was unable to predict growth overcompensation, a phenomenon observed in some crops (Weinig *et al.* 2003, Poveda *et al.* 2010). A clipping experiment in the same essence as the one from Akiyama with a range of leaf removal at different time point would allow testing both qualitatively and quantitatively the predictions drawn from our model. As our model predictions consider stem, flowers and seeds as reproductive mass, it would however be necessary to adjust the methods in order to account for

the whole reproductive mass and not only seed set. Because we modeled herbivory as a one-time event, it may be difficult to draw parallel between the predictions from our model concerning leaf herbivores that tend to stay for long period of time on their host plant. A plant with a herbivore living on it, eating its leaves may not react to this type of perturbation the same way that it would react to a sap-sucker; and the effect is likely to be more pronounced than what the model predicts for a one-time event of herbivory.

Root herbivory has less impact than leaf herbivory

The predicted consequences of root herbivory are similar to the case of leaf herbivory but with a smaller effect on the plant's final reproductive mass. However, it is worth noting that the assumption of the model that root removal occurs during a one-time event is unlikely as root herbivores, generally insects larvae, tends to stay on the roots for extended periods of time (Masters *et al.* 1993, Blossey and Hunt-Joshi 2003). Furthermore, our model assumes a simple relation between nitrogen uptake and root mass and as a consequence allows for a small root to take up all the nitrogen present in the pot. But roots also develop to access nitrogen otherwise unreacheable (O'Brien *et al.* 2007). Therefore damage from root herbivory are likely to be more important than predicted from our model.

The decrease in final reproductive mass from root herbivory is reported in Blossey and Hunt-Joshi (2003) and Poveda *et al.* (2003), with decreases ranging from 0 to 45%. We did not find studies exploring the differences in flowering time due to the type of herbivory, except for Poveda *et al.* (2003) who describe a delay in flowering onset only in the case of leaf herbivory. The findings of Poveda *et al.* (2003) therefore contradict this prediction from the model. A test of the peak N model for root herbivory implies the need to overcome difficulties associated with dealing with roots (Blossey and Hunt-Joshi 2003). A root-removal experiment would be difficult

to carry out in regular soil-settings as it would be difficult to remove a precise percentage of the total root mass without irremediably damaging the plant. We suggest a hydroponic setting for plant growth for this particular experiment. This would allow the experimenter to control both the nitrogen inputs and the root removals precisely.

When should a plant defend itself?

The model presented here suggests that plant tolerance to herbivory is a mechanism that allows plants to largely compensate for the negative effects of herbivory. Furthermore, investment in defence is costly (Mauricio 1998, Agrawal 1998, Strauss *et al.* 2002, Weinig *et al.* 2003, Heidel *et al.* 2004, Dietrich *et al.* 2005, Cipollini 2007, Todesco *et al.* 2010, plus references within Strauss *et al.* 2002). The decrease in fitness caused by defence can be low (Agrawal *et al.* 1999) to moderate (Cipollini 2007). However, the mechanism by which a plant defends itself are numerous (Coley *et al.* 1985) and likely to be the consequences of processes that would be difficult to simplify as a single effect on one plant trait as we did in this chapter, assuming tolerance with respect to plant growth. In this respect, the peak N model is completely different from models linking growth to plant defence such as Coley's and Bryant's (1983). The downside of the peak N model not taking defence into account is that it fails to explain mechanism that would select for the evolution of plant defense. Overall, the growth mechanism used in the peak N model provides a dynamic response to herbivory that seems to correlate with the literature for plant tolerance despite the fact that the peak N model did not account for the possibility of growth overcompensation, a tolerance response to herbivory observed in some annual plant species (Poveda *et al.* 2010).

Concluding Remarks

The peak N model relies on the assumption that nitrogen is provided as a non-renewable pool at the beginning of the growing season, and that the plant can optimise investment in different structures. Here we modified the model to take herbivory into account in simple ways. This reveals that the plant has a built-in tolerance to herbivory due to the way it grows, and can quickly restore an optimal allocation. Because the model is only theoretical, a suite of well-designed experiments are necessary to test the predictions. If the model is proved to be reasonable, it would provide a mechanistic explanation for the plant changes in flowering timing across a variety of situation where herbivory occurs. To our knowledge, there exists no such model of plant tolerance and this instance of the peak N model could therefore fill this gap.

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Chapter 4:

Fitting the Peak N to data on eight ecotypes of *Arabidopsis thaliana*

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Summary

The peak N model predicts the lifecycle of an annual plant under the assumption that belowground nitrogen depletion drives growth. With three physiological parameters, the model predicts allocation to roots and leaves, timing of flowering and leaf senescence. However, the model has yet to be tested against experimental data and compared with predictions from alternative models. In this chapter, we conduct an experiment to test how well plant growth models capture the growth of leaves, roots, and reproductive parts of *Arabidopsis thaliana*. The experiment is designed to estimate bolting time and production of reproductive mass as accurately as possible. We then fit different models to this dataset using the Bayesian inference software Filzbach. We show that the peak N model is only able to capture the lifecycle of the annual plant when certain alterations are made to the original simple model. Those refinements include a soft-switch to flowering, and a modification of the initial mass estimation. The peak N model performed better than models using age- or size-rule for flowering. We conclude that the peak N model is a robust alternative to current models to predict the pattern of growth and timing of flowering in annual plants. We suggest the peak N model could be used routinely for the analysis of other questions relating to plant growth.

Introduction

Flowering time in annual plants is a major event with important fitness consequences (Stearns and Koella 1986, Wada and Takeno 2010, Nord *et al.* 2011). Plants have thus developed fine-tuned mechanisms to ensure that the transition from vegetative to reproductive growth occurs when the right conditions are met (Bernier *et al.* 1993, Levy and Dean 1998, Boss *et al.* 2004). For the past twenty years, studies on the plant *Arabidopsis thaliana* uncovered the complex regulatory pathways that trigger flowering at the molecular level (Guo *et al.* 1998, Sheldon *et al.* 2000, Cerdan and Chory 2003, Blázquez *et al.* 2003, Komeda 2004, de Montaigu *et al.* 2010). At first approximation, one can describe flowering as a mechanism actively repressed by flowering regulators that release their grip once temperature, photoperiod, and other physiological and environmental cues have reached the appropriate level, determined through time by evolutionary forces (Cohen 1976, Koornneef *et al.* 1998, Simpson and Dean 2002, Metcalf *et al.* 2008). Recently, Wahl *et al.* (2013) showed that the regulatory pathway involved in the plant nutritional status needed to be fulfilled first before other flowering regulatory pathways.

These molecular mechanisms translate at the phenotypical level into life-history strategies that differ according to environmental conditions and the constraints inherited through evolutionary history (Satake 2010). An annual plant invests in vegetative tissues for as long as it yields higher fitness return than reproductive tissues (Iwasa 2000). The classical interpretation for flowering time optimisation is that the plant has to delay flowering as long as possible in order to gather the largest amount of material to build a large seed set (Abrahamson and Gadgil 1973, King and Roughgarden 1982, MitchellOlds 1996). Environmental conditions and the plant physiological characteristics set the limit for flowering time, creating the variation in timing of flowering in the

process (Stinchcombe *et al.* 2004). Plants therefore flower as late as possible or when they are as big as possible, according to the optimum theory. This leads to the existence of two distinct rules for flowering: an age rule and a size rule (King and Roughgarden 1983, Lotz 1990, Lewis *et al.* 2003, Bolmgren and D. Cowan 2008).

A plant following a size rule for flowering will only flower once it has reached a certain size - or mass - while a plant following an age rule will not flower until a certain delay past germination. From both rules, it is expected that plants flowering later have higher fitness as they have grown vegetatively for longer and are therefore bigger (Koornneef *et al.* 1998). The variation in flowering time among a set of ecotypes would reflect variation in local optima that arose from selection (Alonso-Blanco *et al.* 1998).

For a set of ecotypes grown in similar conditions, we would expect the following outcomes for a species following a size rule: plants of the same ecotype should flower at the same size and there would be significant variation in size at flowering. Ecotypes flowering later should produce more seeds than ecotypes flowering earlier. Similarly, plants growing according to an age rule would flower at the same time regardless of the size with significant variations in age at flowering; however ecotypes flowering later should also produce more seeds than ecotypes flowering earlier.

We recently proposed an alternative to these rules with the peak N model (Chapter 1;Guilbaud *et al.* 2014, in review). This model relates environmental conditions to the growth rate of an annual plant, assuming belowground resources are limiting and the plant seeks to keep balance between growth rates of above- and below-ground tissues. In theory, the peak N model can predict the allocation to roots and leaves, the switch from vegetative growth to reproductive growth (i.e.: the

flowering time), and the senescence of leaves. To achieve this, the model is given three physiological parameters: the carbon-nitrogen ratio, the leaf photosynthetic efficiency and the root nitrogen uptake efficiency. It is called peak N because it predicts a plant should flower when its nitrogen uptake is maximised. Accounting for the relevance of our model can be complicated as there is no straightforward way to relate parameters such as the root uptake rate to typical plant growth experiment data; masses of roots and leaves, timing of germination, of bolting, or the final reproductive output. Fortunately, Bayesian methods, through well-defined routines (Buckland *et al.* 1997, Johnson and Omland 2004, Posada and Buckley 2004), give the possibility to fit the model to data and therefore provide both insight into the biological relevance of the peak N model and a fit to experimental data. Another difference between the peak N model and the two aforementioned rules is that the peak N hypothesis does not directly equates timing of flowering with fitness as it leaves open a spectrum of different post flowering strategies with different possible fitness outcomes (chapter 2). These different post-flowering strategies rely on the allocation of growth to different plant parts after the initiation of flowering.

In the previous chapters, we described a model of growth for an annual plant (chapter 1), explored the assumptions of the model (chapter 2), and extended its potential range of application to biotic interactions (chapter 2-3). However, we have not yet tested the peak N model against experimental data.

In this chapter, we use data from an experiment specifically designed to test the peak N model and fit the model to these data. The main difference between this experiment and regular growth experiment is the attention paid to bolting time, the building of reproductive mass, and the changes in size through time. The fit of the model to the data is accomplished using Filzbach, a tool developed to simplify the use of Bayesian inference in the context of ecological studies.

The dataset presented evidence that led us to modify the peak N model. We then tested annual plant growth models with different rules for flowering and compare their predictions to the data. We compared the likelihood of the different post-flowering strategies possible with the peak N model given our data. We show that the peak N model provides a better fit to the data than the tested alternatives, that it can account for differences in physiological parameters among a set of *Arabidopsis* ecotypes and that some further refinements to the model can improve the fit to data even further.

Materials and Methods

The experiment

We grew eight ecotypes of *Arabidopsis thaliana* (ma-0, col-0, nw-0, no-0, sap-0, ll-0, ru-0, and kin-0) and let them perform a complete lifecycle. We chose the ecotypes according to their flowering day assessed in controlled conditions in a previous experiment (Zuest *et al.* 2012, table.4.1). In the original experiment, seven out of eight ecotypes had an average flowering day in the range of 14.8 to 18.0 days and one ecotype, ll-0, flowered late after 34.3 days on average.

Ecotype	Mean bolting age
ma-0	14,8 (1)
no-0	15,0 (2)
col-0	16,3 (3)
nw-0	16,4 (4)
ru-0	16,8 (5)
sap-0	17,4 (6)
kin-0	18,0 (7)
ll-0	34,3(8)

Table.4.1. Ecotypes and their mean bolting age (From Zuest *et al.*, 2012). Bolting order in parenthesis.

The plants were grown in a climate chamber at 21 degrees Celsius in long-day conditions (16 hour light) and were watered regularly. We mixed nutrient-poor mineral soils with nutrient-rich germinating soil in the proportion 4:1 (80%-20%) to create low-nutrient growing conditions. We sowed three seeds per pot in individual cells, and then trays were wrapped in plastic foil and kept at 6 degrees Celsius during four days for stratification. After plastic foils were removed (when all plants had two leaves), supernumerary individuals were removed to leave one individual per pot, the healthiest. We harvested plants according to the following schedule: 2 harvests took place before bolting (12 and 18 days after germination) one took place on the day of bolting and the following harvests were performed according to the bolting day. We collected plants on bolting day +1, +2, +3, +5, +7, +9, +11, +14, +17 (see table.4.2).

Harvest number	Day after sowing	Day after bolting	Compartment collected
1	20	-11	Leaves and roots
2	26-28	-5	Leaves and roots
3	23-49	0	Leaves and roots
4	22-51	1	Leaves and stem(s)
5	25-50	2	Leaves and stem(s)
6	24-46	3	Leaves and stem(s)
7	28-55	5	Leaves and stem(s)
8	31-46	7	Leaves and stem(s)
9	32-54	9	Leaves and stem(s)
10	35-54	11	Leaves and stem(s)
11	37-53	14	Leaves and stem(s)
12	40-53	17	Leaves, stems and roots
13	111	55-80	Leaves, stems, roots and seeds

Table.4.2. Schedule of destructive harvests. Differences in days after sowing or bolting appear from differences in germination time or bolting time or both.

Finally, we waited until the whole plant had senesced to carry out the last harvest (111 days after sowing). There were thus 13 harvests. We followed this schedule for harvests in order to maximise the collection of information around bolting day –the time when the mass should be changing most quickly. At each harvest, we weighted leaf mass, and stem mass if present. Root mass was assessed on harvests 1, 2, 3, 12 and 13 (two early harvests before bolting day, one on bolting day, and two late harvests after bolting day). Two weeks prior to the final harvest, we taped the plants stems together and enclosed them in paper bags in order to passively collect

seeds. On the final harvest, seed mass was collected from the bag and from the senesced stems. We weighed a 100 seeds on four replicates of each ecotype to estimate the average seed mass. This value of seed mass (*Seedmass*, see S.4.A) is later used to initialise the growth models. At each harvest, we sacrificed four replicates of each ecotype. Because of bolting asynchrony, replicates from the same harvest may have been collected on different dates. In total, we grew 416 plants.

CHN analysis

To assess a plant C:N ratio in different plant parts and to track whether this ratio changes through time, we performed CHN analysis on 80 selected individuals at different points of the lifecycle. A CHN analysis allows to determine the proportion of carbon, nitrogen and hydrogen in an organic sample, hence the name (Gnaiger and Bitterlich 1984). We selected individuals from each ecotypes from harvest 1, 2, and 3 (before bolting and on bolting day) from which we assessed leaf and root C:N ratios. From harvest 12 (bolting day +17), we assessed the C:N ratio of leaves, roots, and the reproductive stem (bearing flowers and siliques) of one individual per ecotype. We then assessed the C:N ratio of all plants parts including seeds from the final harvest. In order to gather sufficient mass to perform the analysis, several plants were pooled together for the earlier harvests when plants were small. In total for each of eight ecotypes, we collected one sample of seed, one sample of stem, four samples of leaves and four samples of roots.

Model modification

Two observations from the dataset led us to modify the original peak N model and its competitor models. First, early plant growth rate was too high for the model to estimate the trend over the rest of the lifecycle with fixed parameters from the given seed mass. To deal with this issue, we

added a parameter, *initial_mass*, to the model that is a multiplier of the weighted seed mass. As a result, the first iteration of the model run uses *Seedmass*initial_mass* instead of *Seedmass* alone (S.4.A). During the fitting, we allow *initial_mass* to take a wide range of possible values, from 1 to 1000, so that the estimation of other parameters does not rely heavily on the starting values. The second observation was that we observed vegetative growth after the plant has bolted. Because –unlike the original peak N model – data says there is no "hard-switch" between vegetative and reproductive growth, we implemented a "soft-switch" that simply consists in a progressive investment in reproductive growth centered around peak N. This progressive switch follows a logistic function and requires the addition of an extra parameter, ε, which accounts for the time it takes for the switch to occur (S.4.A). At any given time t, the fraction *frac_flower* allocated to reproductive tissues is:

$$frac\_flower = \frac{1}{1+e^{-\frac{t-t_{inf}}{\varepsilon}}} \quad \text{(Eqn.4.1)}$$

Hence, the smaller the value of ε, the faster the switch occurs. At the time the plant reaches peak N (t_{inf}, see eqn.S.1.11 from Chapter 1), 50% of the plant growth is diverted to reproduction. This modification implies that the plant produces some reproductive mass from germination. Increased initial mass and a soft-switch to flowering are implemented in all competing models therefore models differences in their ability to predict data cannot be due to these modifications. We discuss the implications of both issues later.

Filzbach

The peak N model was coded in C++ and tested with the Bayesian inference software Filzbach. Code for the model and all alternatives is provided as supplementary material (S.4.A, the code requires Visual Studio C++ or a similar compiler). Filzbach (http://research.microsoft.com/en-

us/um/cambridge/groups/science/tools/filzbach/filzbach.htm) is a tool developed to perform parameter estimation of models and model selection. The implementation consists mainly in writing the model and defining the likelihood function. The likelihood function takes our experimental data, runs the model for a random assemblage of parameters and compares the probability of observing the data for this set of parameters. This routine is performed many times to estimate the set of parameters that is the most likely to provide the observed data. Once the fitting is accomplished, we can relate the likelihood of the parameter values estimated for this dataset to the probability of observing the dataset given the parameter values thanks to the likelihood function:

$$L(S/x) = P(x/S) \qquad \text{(Eqn.4.2)}$$

Where S is the dataset and x the set of parameters used to fit the model. Because we do not have the same number of data points for different plant parts, we weighted the likelihood function in order for each compartment to have the same importance. This ensures that the software does not try to fit the model to one plant part at the expense of another. Finally, each parameter is given a starting value, and a range of possible values. We took a wide range of possible values for each parameter to ensure the likelihood calculation does not end up trapped at a local maximum. We ran Filzbach for 20,000 iterations in the burn-in phase and 20,000 iterations in the sampling phase. This routine is accomplished with 3 chains and we present results from the best performing chain, the one with the highest log-likelihood. Each chain starts from a different random value which also ensures that the likelihood calculation is not trapped at a local maximum. The software outputs parameter estimation with confidence intervals, log-likelihood, Akaike Information Criteria (AIC), and predictions for masses and bolting day (estimated as the day the plant reaches peak N). We used parameter estimation from the software to estimate the

accuracy of the model predictions compared with the experimental data. Akaike Information Criteria and log likelihoods were used to compare the simple model with i) ecotype-specific models, ii) refined versions of the simple peak N model iii) models with alternative rules for flowering and iv) models with alternative rules for the production of reproductive mass.

Ecotype specific models

The simple peak N model estimates a single set of parameters, which are shared by all ecotypes. However, each ecotype may have undergone selection over any of the model parameters and we would thus expect each ecotype to behave slightly differently, and hence their physiological parameters should be different. To account for this in Filzbach, we allow each parameter to be ecotype specific. In practice, each of the three physiological parameters of the model is allowed to have one value per ecotype instead of a global value for all ecotypes. As a result, Filzbach returns predictions specific for each ecotype. We ran the program with one, two, or all three physiological parameters ecotype-specific for a total of seven ecotype-specific models. Notice that the parameters for initial mass (*initial_mass*) and for the gradual reproductive switch (ε) remain global. Furthermore, when defining parameters as ecotype-specific, each parameter in the new set of ecotype-specific parameter is evaluated with the subset of data available for the specific ecotype. Those models are later referred to as ecotype-specific models.

Refinements of the model

The peak N model is built with many simplifying assumptions. These are useful from a conceptual point of view but may translate poorly in reality. We refined the model in two ways: first by adding a cost for translocation and second by allowing for different C:N ratios in different plant parts. The model assumes perfect translocation of C from leaves to reproductive

mass, which is unlikely to be the case in reality, therefore we accounted for carbon transfer inefficiency by adding a new parameter. The translocation efficiency parameter *T* varies between 0 and 1 and account for the amount of mass effectively transferred from leaves to reproductive mass. Thus, after bolting:

$$M_{Leaf,t+1} = M_{Leaf,t} - \frac{\rho}{\gamma} G_{root,t} \quad \text{(Eqn.4.3a)}$$

$$M_{rep,t+1} = M_{rep,t} + T\frac{\rho}{\gamma} G_{root,t} \quad \text{(Eqn.4.3b)}$$

While the first equation (Eqn.4.3a) remains the same as in the original model, the second (Eqn.4.3b) has the translocation parameter *T* added.

We previously considered the C:N ratio (ρ) to have the same value in all plant parts and it appeared to be inaccurate from the CHN analysis, we thus relaxed this assumption, by allowing for different C:N ratios for each compartment. The upper and lower range of potential values for the C:N ratio in a given plant part was defined using the data from the CHN analysis; however, Filzbach does not fit the predicted C:N ratios directly from CHN data. Adding different C:N ratios for each compartment implies model modifications of the equation describing nitrogen depletion (Eqn.4.4) and the calculation of root growth (Eqn.4.5).

$$N_t = N_0 - \frac{M_{leaf,t}}{\rho_{leaf} V} - \frac{M_{root,t}}{\rho_{\mathrm{root}} V} - \frac{M_{flower,t}}{\rho_{\mathrm{flower}} V} - \frac{M_{seed}}{\rho_{\mathrm{seed}} V} \quad \text{(Eqn.4.4)}$$

$$G_{root,t} = \rho_{\mathrm{root}} \theta N_t M_{root,t} \quad \text{(Eqn.4.5)}$$

In equation 4.5, we use the root ρ_{root} C:N ratio to define the root potential growth. This implies we assume the balance of carbon and nitrogen in the roots is to be maintained at the expense of the balance in the leaves. Alternatively, equation 4.5 could be written using the leaves C:N ratio.

We chose to use root C:N ratio over leaf C:N ratio to remain consistent with the assumption that the plant grows in a nitrogen-limited environment. This change affects the initial mass repartition (S.1.B.1, it now depends on the seed C:N ratio), the calculation for time to inflection (depends on root C:N ratio by choice), and the final mass of the plant (S.1.11 and S.4.A, it depends on C:N ratios of all plant parts).

Adding a translocation cost or different carbon-nitrogen ratios for different plant parts do not contradict the structure of the peak N model. Their implementation however allows taking into account some reality of what plants have to deal with during their growth.

Implementation of alternative flowering rules

The two alternative flowering rules with which we fit the data are an age-based rule and a size-based rule. In the case of the age rule, we added a free parameter, *Day*, allowing the plant to flower at any time during the growing season. This parameter is set ecotype specific in order to test the assumptions that if a plant follows an age rule, individuals of different ecotypes would flower at significantly different times and ecotypes flowering later would produce more seeds.

In the case of a size rule, we added a free parameter, *Size*, that is used in the calculation for time to inflection (following equation S.1.8) and therefore the expected size at flowering. This parameter is also set to be specific for each ecotype to test for variation in size at flowering and seed output among ecotypes. These two alternatives are different from the peak N model in essence. Using Filzbach, we compare how well the three rules for flowering explain the data.

Implementation of alternative post-flowering strategies

Finally, we test how well different post-flowering strategies of the peak N model fit to our data. The alternative post-flowering strategies we chose are the same as in chapter 2. In all models presented so far, it is assumed that a flowering plant allocates as much new growth as possible to reproduction including translocating excess leaf mass to reproductive mass. This strategy is the "all-for-flower" strategy that is, in theory, optimal (chapter 2). The four post-flowering alternative strategies are the following: the "photosynthetic competitor" uses newly produced photosynthates to grow reproductive mass and translocates leaf mass into roots to maintain a high nitrogen uptake. The "photosynthetic flowerer" does not translocate leaf mass and produces reproductive mass only through photosynthesis. The "translocative competitor" produces reproductive mass via translocation while investing photosynthates in roots and finally, the "translocative flowerer" grow reproductive mass only through translocation (implicitly stopping photosynthetic activity).

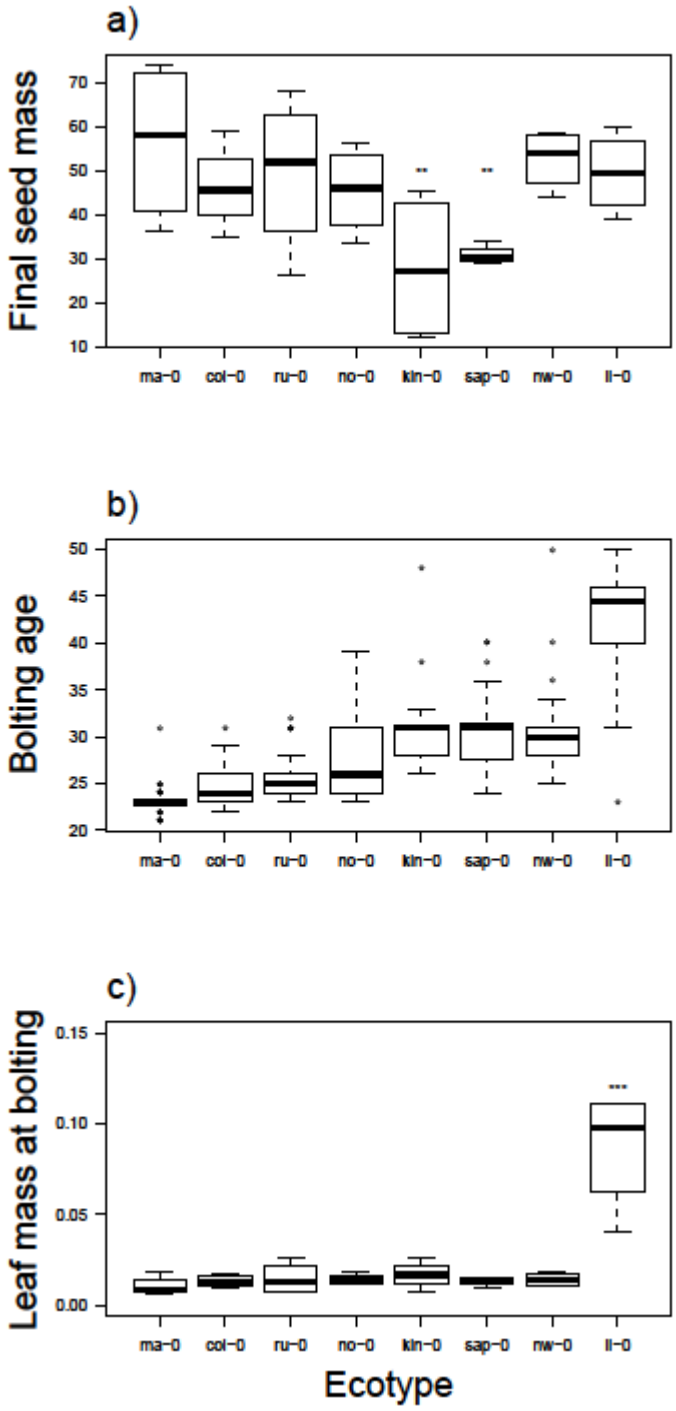

Figure.4.1. Performance of *Arabidopsis thaliana* ecotype grown in nutrient-poor conditions. a) the seed mass collected at the end of the experiment when plants had completely senesced. b) The recorded bolting time of each ecotype. Bolting is the appearance of the first stem bud. c) Recorded leaf mass at bolting. Ecotypes are ordered by bolting time.

Results

All ecotypes grown in poor soil bolted significantly later than in Zuest's experiment (t = -3.4178, df = 13.96, p-value = 0.004178). Plants bolted on average 10.7 days later in our experiment. The order of bolting also changed (table.4.3) although ma-0 still bolted the fastest while ll-0 remained the latest bolting ecotype. The general delay of flowering might be an effect of the nitrogen-poor growing conditions.

Ecotype	Mean bolting age
ma-0	23.1 (1)
no-0	27.7 (4)
col-0	24.7 (2)
nw-0	30.2 (6)
ru-0	25.7 (3)
sap-0	30.3 (7)
kin-0	30.1 (5)
ll-0	42.8 (8)

Table.4.3. Ecotypes and their mean bolting age in the current experiment. Bolting order is in parenthesis.

To test the assumption that later bolting is associated with higher reproductive output, we compared the final seed mass of all ecotypes. In our experiment, each ecotype bolted at significantly different age ($F_{7,344}$=119.9, p< 2.2e-16) and all ecotypes but ll-0 flowered at similar leaf mass (Figure.4.1b,c; $F_{7,24}$=16.27, p=1.053e-7) whereas reproductive output was similar for most ecotypes (Figure.4.1a; $F_{7,24}$=2.569, p=0.03985). Only kin-0 and sap-0, both rather late ecotypes, had significantly lower reproductive output (t=-3.194, p=0.0039 and t=-2.869, p=0.00845, for kin-0 and sap-0 respectively). As a result, our data suggests that later bolting may in fact be associated with lower reproductive output ($F_{1,30}$=4.6987, p=0.03825, Figure.4.2). This is not an uncommon result in experiment dealing with low nutrients (Paul-Victor *et al.* 2010, Zuest *et al.* 2012).

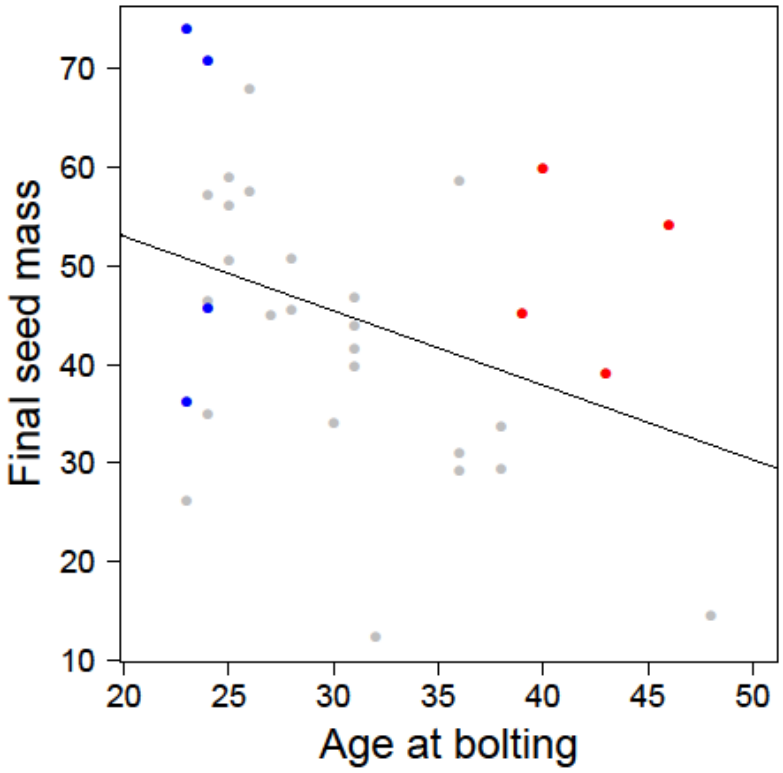

Figure.4.2. Final seed mass versus age of bolting for eight ecotypes of *Arabidopsis thaliana*. Late bolting ecotype (ll-0, red dots) did not produce more seeds than early bolting ecotype (ma-0, blue dots). The black line represents the correlation between both factors: r=-0.36.

C:N ratios

Contrary to the original assumption of the model, the C:N ratio differed in all plant parts. This ratio also varied through time. The C:N ratio in seeds and flowers was low compared to leaves and roots (mean seed C:N ratio=19.51; mean flowers C:N ratio=11.67). The low C:N ratio in seeds and flower is a result of higher nitrogen content compared to roots or leaves (nitrogen constitute 3.88% of seeds and 2.91% of flowers on average). In leaves and roots, where four time points are available, it is possible to track the dynamic through time. In leaves, the ratio started low and increased through time (Figure.4.3a). A steep increase is observed around the time of

bolting. The increase is a consequence of the decrease in nitrogen content (mean leaf nitrogen content at t=-5 is 4.26%; at t=0, leaf nitrogen content is 1.52% which is 2.8 times less). A steep decrease in nitrogen content may reflect that the plant becomes nitrogen-limited, which would be in agreement with the peak N model. Root C:N ratio is high throughout the season (Figure.4.3b), mainly because roots contain less nitrogen than the leaves (leaves contain 2.96% nitrogen on average, while roots contain 0.94%. the difference is significant: t = 6.2607, df = 32.072, p = 5.065e-07). All ecotypes behaved in a similar fashion, with a steep increase in leaf C:N ratio around bolting time and a high, generally increasing, root C:N ratio.

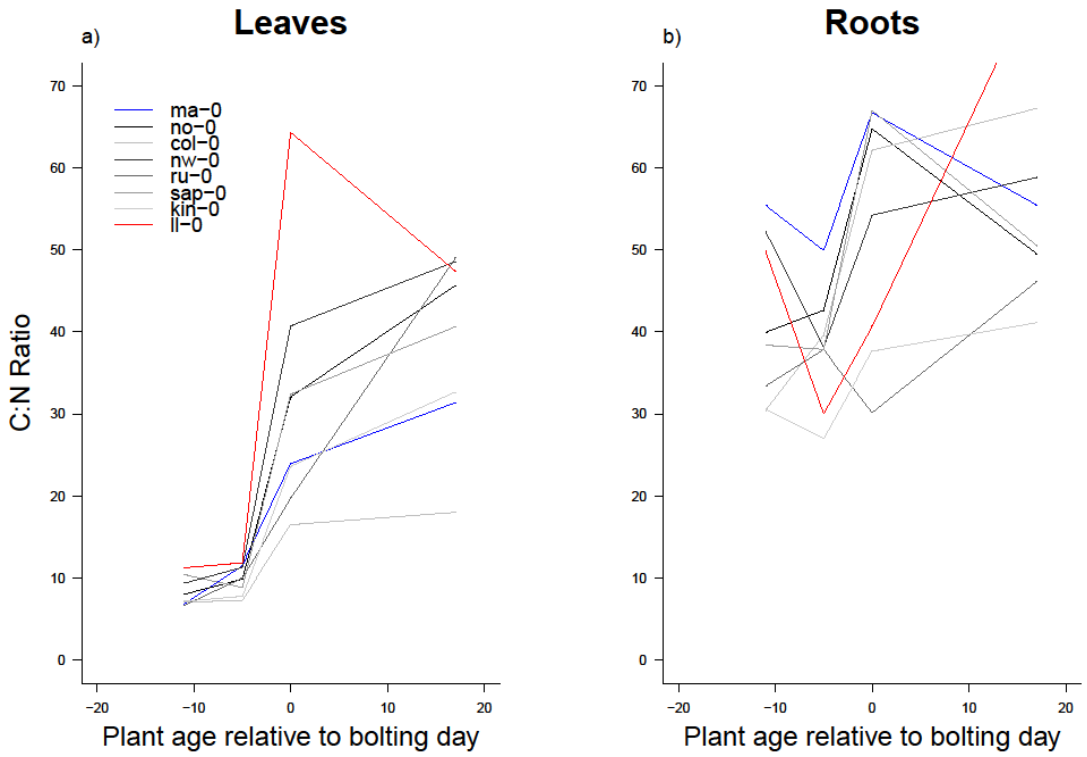

Figure.4.3.C:N ratios in a) leaves and b) roots for eight ecotypes of *Arabidopsis thaliana* grown in nitrogen-poor conditions. C:N ratio is estimated at four time points during the experiment to observe the dynamic of carbon to nitrogen ratio through time. In leaves, a steep increase in C:N ratio occurs around bolting day. The red line corresponds to the late-flowering ecotype while the blue line corresponds to the ecotype that bolted the earliest.

Fitting of the peak N model

The peak N model with all three physiological parameters shared among ecotypes returned a log-likelihood value of -270.19 (AIC=558.39, Table.4.4). The Bayesian algorithm estimated the following values for the three physiological parameters: mean leaf photosynthetic efficiency γ=0.19; mean root nitrogen uptake rate θ=0.087; mean C:N ratio ρ =37.11. Using the mean for the parameters value, the model also predicts individual size at the time of harvest for roots, leaves and reproductive mass when present. Parameters values are also used to estimate the time the plant reaches peak N. Using the predicted masses provided by Filzbach, we compared predicted mass with experimental data (Figure.4.4a-c). With linear regressions in R, we found that root mass was best predicted with an r^2=0.85 (Figure.4.4d) followed by leaf mass (r^2=0.76) and finally reproductive mass (r^2=0.69). In the case of a peak N model with a single set of parameter across ecotypes, the time to switch from vegetative growth to reproductive growth, defined as 2ε, is 22.92 days. This means the plant initiated investment in reproductive tissues more than 11 days before the peak of nitrogen uptake, and it kept producing a noticeable amount of vegetative tissues long after the plant flowered. The switch is therefore quite smooth as it is predicted to last 20.6 % of the total duration of the experiment. Generally, the model captured accurately the mean masses for leaves and roots but overpredicted final reproductive mass (Figure.4.4c). The reason for this overprediction can occur in part because of the lack of data between day 60 and 111 in the experiment, as plant were left to senesce, and the difference in number of data points at the end of the experiment compared to earlier stages. Adding data for later reproductive tissues production would enhance the fit accomplished by the Bayesian algorithm when it computes likelihood for reproductive tissues because it would spend less sampling iteration optimizing prediction for early reproductive tissues. Another reason that might

equally explain the overprediction of final reproductive mass is that reproductive tissues may not be able to grow as well as vegetative tissues (their growth rate is lower) because of different physiological properties of reproductive tissues compared with other tissues.

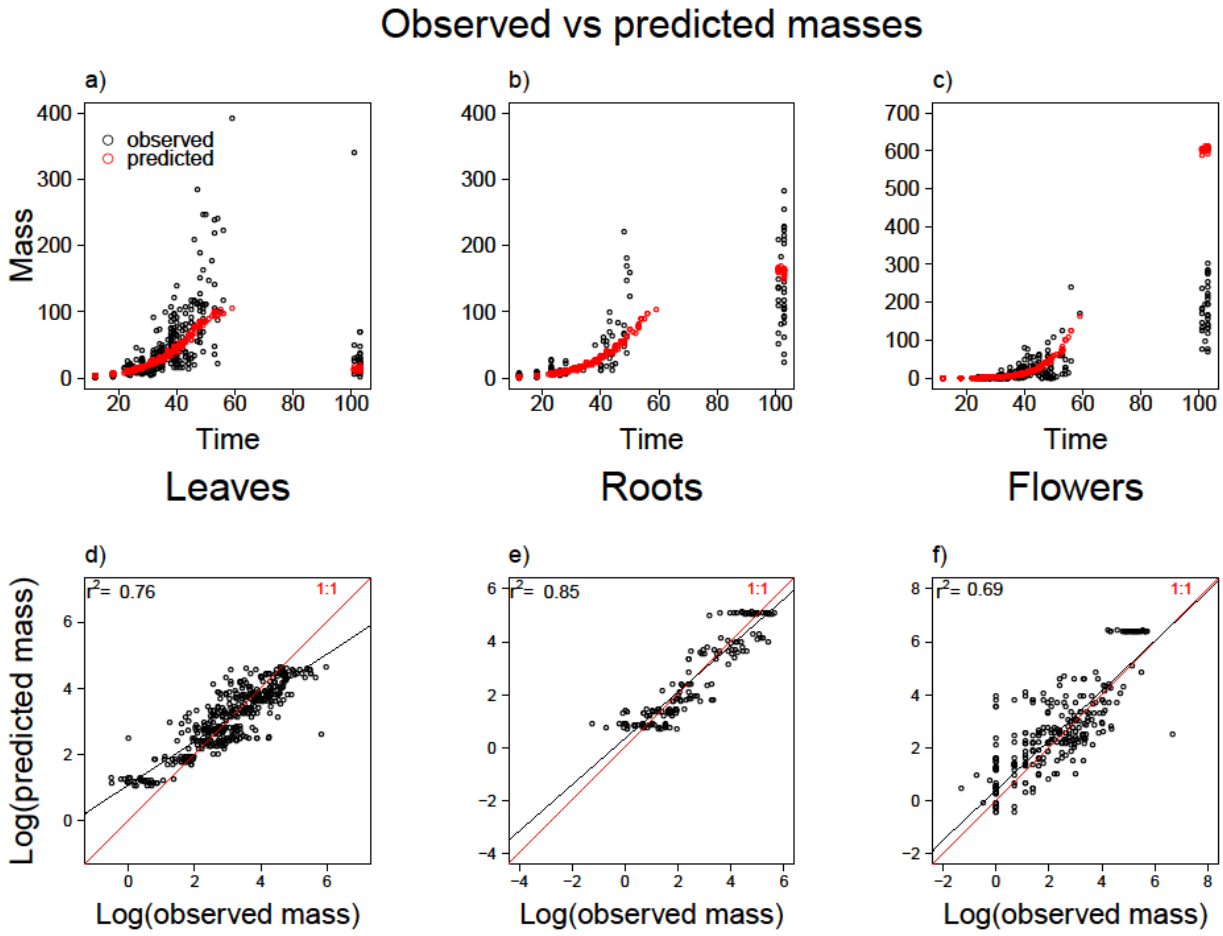

Figure.4.4. Comparison between model prediction and experimental data. Panels a, b and c present the changes in mass through time for the three compartments predicted from the global model (red dots) with the observed data (black dots). Panels d, e, and f represent the correlation between predictions and observations put on a log scale to avoid heteroscedasticity. Predicted data are provided as output from Filzbach and are computed for each individual during the fitting. Because the predictions come from a model where parameters are shared among ecotypes, the variation in predicted mass only appears from the variation in initial seed mass and the time of germination.

Fitting of ecotype-specific models

By allowing the model to estimate one or more physiological parameter as ecotype-specific, we improved the likelihood. Among the ecotype-specific models, the one achieving the best balance between complexity and goodness of fit was the model with the photosynthetic leaf efficiency γ

set as ecotype-specific (log-likelihood=-260.43; AIC=554.86, Table.4.4, Figure.4.6). The model with nitrogen uptake rate set as ecotype-specific was equivalent (log-likelihood=-261.14), while the model with the C:N ratio ρ ecotype-specific was worst (log-likelihood=-265.96). Finally, the model with all three parameters set as ecotype-specific returned the best likelihood (log-likelihood=-257.11) at the expense of added complexity (AIC=580.23). In all cases, overprediction of the final leaf mass persists.

Model Type	Number of parameters	Log(Likelihood)	AIC
Global peak N model	3	-270.19	558.39
Peak N model γ ecotype specific	10	-260.43	554.86
Peak N model ρ ecotype specific	10	-265.96	565.92
Peak N model θ ecotype specific	10	-261.14	556.27
Peak N model $\theta + \rho$ ecotype specific	18	-258.77	567.47
Peak N model $\theta + \gamma$ ecotype specific	18	-260.18	570.37
Peak N model $\rho + \gamma$ ecotype specific	18	-258.73	567.47
Peak N model all parameters ecotype specific	24	**-257.11**	580.23
Translocation	4	-266.64	553.28
Different C:N ratios	6	-264.12	**552.25**
Age-rule	11	-268.10	562.53
Size-rule	11	-334.22	704.44
Photosynthetic competitor	3	-283.87	585.75
Photosynthetic flowerer	3	-274.92	567.85
Translocative competitor	3	-463.49	942.72
Translocative flowerer	3	-462.36	944.99

Table.4.4. Goodness of fit for all the models tested.

Age-rule and size rule for flowering

We used the model with γ set as ecotype-specific to compare the ability of the different model to predict bolting time. A model using an age rule for flowering rather than a peak N rule returns an AIC of 562.53 and the model with a size rule for flowering returns an AIC of 704.44. An age-rule for flowering is slightly worse than the peak N rule while a size rule does not fit our data for bolting. When comparing for the ability of all three model to predict bolting time, we observe that the age-rule predicts time to flower better than the peak N model (r^2=0.57 compared to r^2=0.51 for the peak N rule). However, this improved prediction comes at the expense of an extra parameter, *Day*, whereas timing of flowering with the peak N rule arise from the physiological functioning of the plant. However, when using the most complex peak N model with all parameters set as ecotype-specific, the prediction of bolting time correlates with an r^2 of 0.61. Both the age-rule and the peak N rule predict bolting to occur several days later than observed (Figure.4.5a-c). The delay between observed bolting and predicted peak N is a consequence of the soft switch to flowering: in both cases, the predicted time of bolting corresponds to the moment half of the growth is invested in reproduction, as described in the method part.

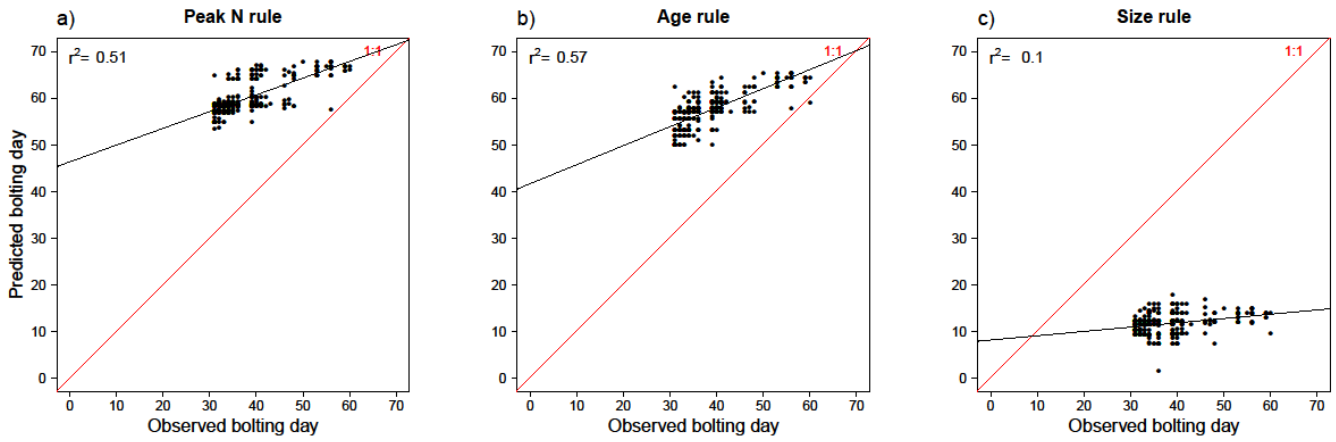

Figure.4.5. Predicted vs. observed bolting day for the three different rules for flowering. Predicted bolting time corresponds to the time when 50% of growth is allocated to reproduction. For the peak N rule a) this corresponds to peak N. It is determined with an ecotype-specific parameter Day or Size for the competing rules (b and c). For the peak N rule, we selected the model with γ set as ecotype specific in order to compare models with one parameter set as ecotype specific.

Refinements of the model

Adding a cost to translocation or using different C:N ratios in different plant parts improved the fit compared to the simple peak N model (Table.4.4). The translocation efficiency parameter *T* was estimated to be as low as 0.04, this means that our model predicts that less than 4% of leaf mass is successfully translocated to the reproductive part of the plant. With a low translocation, the final predicted reproductive mass was lowered (mean final predicted reproductive mass is 507.62 mg vs. 604.51 mg in the global model) but remained very high compared to observations (180.55 mg).

Using different values of C:N ratios for different plant parts also improved the fit. The model predicted low value of C:N in seeds and reproductive parts and higher value of C to N in roots, in accordance to the data. The predicted C:N ratio in seeds is 12.02 compared to 19.51 the mean of

seed C:N ratio from our data, the predicted C:N ratio in reproductive parts is 6.97 compared to 11.67 from the data. In roots, the predicted C:N ratio was 1.84 times lower than observed (22.52 vs 41.61). Finally, the leaf C:N ratio was worst predicted (1.58 vs 21.69; 13.72 times lower than observed). The inaccuracy of the prediction may be the consequence of a static C:N ratio in the model compared to the dynamic change that our analysis showed.

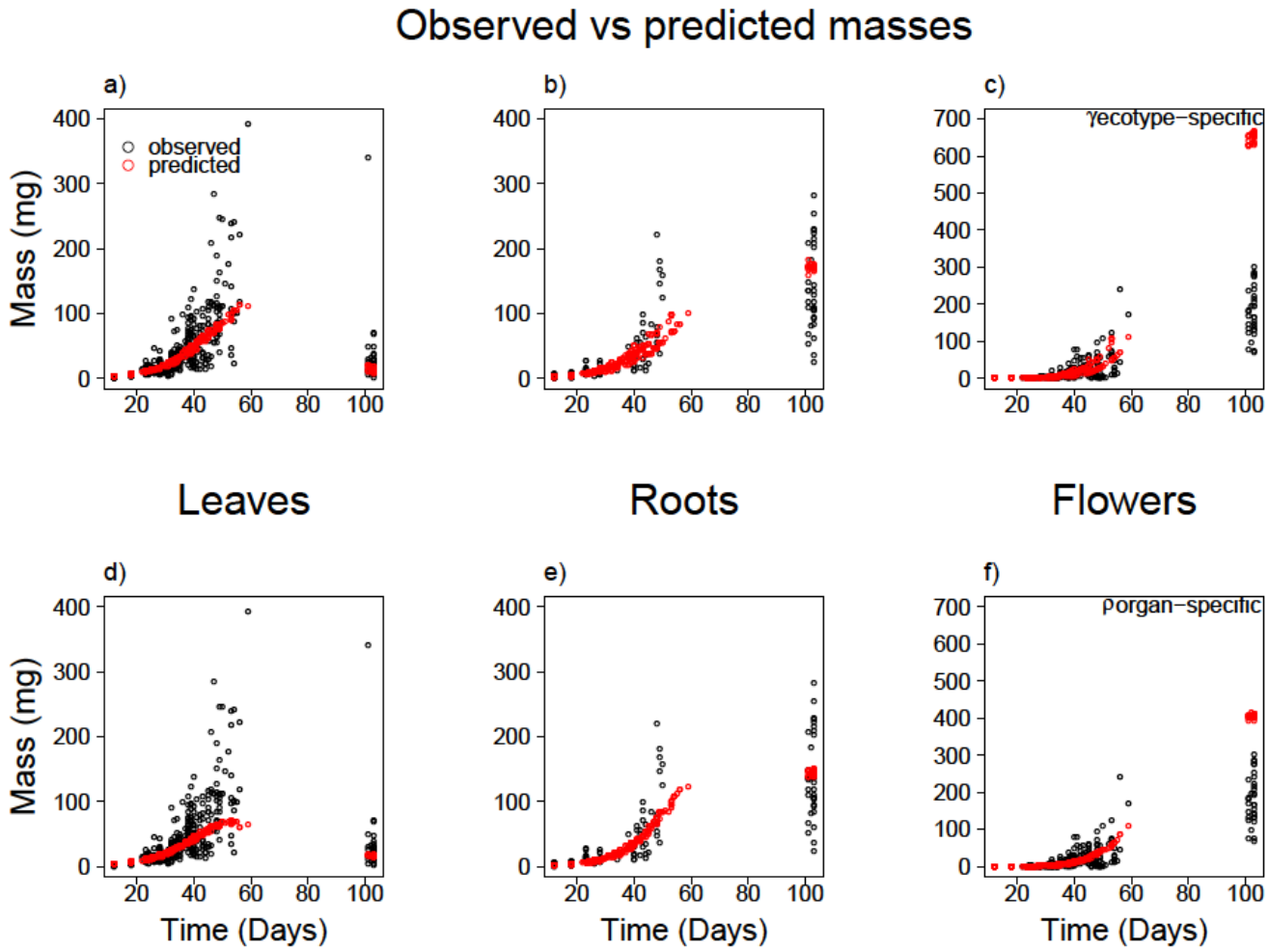

Figure.4.6. Comparison between model prediction and experimental data for the model with γ set as ecotype-specific (a,b,c) and for the model with C:N ratio set as organ-specific (d,e,f). Predicted data are provided as output from Filzbach and are computed for each individual during the fitting. The final reproductive mass in both model is predicted to be higher than observation (c,f). The model with organ-specific C:N ratios predicts has the lowest overprediction.

Alternative post-flowering strategies

Finally, we compared the likelihood and AIC of the simple peak N model with models using alternative rules for reproductive part production. Among these strategies, the ones relying on translocation for reproductive tissues production, the translocative competitor and the translocative flowerer, performed poorly compared to the ones relying on photosynthesis (Table.4.4). Strategies with continued photosynthesis after flowering, photosynthetic competitor and photosynthetic flowerer, had lower likelihood and higher AIC than the "all-for-flower" strategy implemented in the original peak N model hence this latter strategy is better than the tested alternatives.

Discussion

The main reason for developing the model was the paradoxical observation that, if an annual plant uses age-rule for flowering, flowering day could not be delayed e.g.: by short day/long day shifts (Hayama and Coupland 2003, Yanovsky and Kay 2003, de Montaigu *et al.* 2010). Similarly, the plant could not either use a size-based rule because fertilisation -or pot size- increases plant size at flowering (Sønsteby *et al.* 2009,Bolmgren and Cowan 2008, Akiyama and Ågren 2012, Agren *et al.* 2012, Poorter *et al.* 2012). The peak N model was developed with the goal of reconciling both observations through the rule that plants flower when they reach the maximum uptake rate, or at least initiate flowering according to this peak. Yet, the peak N model remained untested and largely theoretical. With this experiment and consequent fitting to data, we show that the peak N model predicts the life cycle of *Arabidopsis thaliana* with satisfactory accuracy for the vegetative growth although it predicts too high reproductive outputs.

Age vs size vs peak N

The peak N model showed to fit data better than model with age- or size-rule for flowering. A model with one parameter ecotype-specific provided a better log-likelihood for a less complex model (Table.4.4). Ultimately, the peak N model differs from its competitor models because it does not assume later bolting to be associated with increase reproductive output. With the growth condition used in our experiment, it seems that this largely used assumption (King and Roughgarden 1983, Koornneef *et al.* 1998, Iwasa 2000, Roux *et al.* 2006) does not hold. The relationship between time of flowering and reproductive output may hold only in nitrogen-rich conditions. While the mechanism that permits *Arabidopsis* to assess the optimal time for flowering may be complex (Simpson *et al.* 1999, Boss *et al.* 2004) and might have several "checkpoints" before flowering initiation (Wahl *et al.* 2013), peak N might be a unifying concept. A more molecular-based approach would be necessary at this point to confirm the insight our ecological model provides.

Flowering transition and initial mass

It was necessary to introduce a smooth transition from vegetative growth to reproductive growth because it appeared clearly from the data that vegetative growth still took place after bolting. For practical reasons, this smooth transition mathematically assumes that growth is allocated to reproduction from the beginning of the season which may appear to contradict the peak N rule. However, the consistent delay between predicted peak N and observed bolting day suggests that the plant indeed invests in reproductive structures long before peak N. The correlation between Peak N and bolting is high ($r^2 = 0.61$ for the model with all parameter set as ecotype specific), suggesting that *Arabidopsis thaliana* does not flower "at" peak N, but "according to" peak N.

The plant estimated ε for the global model is 11.46 days, meaning that it takes roughly 11 days for the plant to initiate flowering and reach 50% of growth allocated to reproduction. It then takes 11 other days to stop growing vegetative structures. It is important to remember that peak N is an emergent property of the physiological parameters, which drive growth and not flowering time. As we did not set constraints on flowering time in the algorithm, peak N is very sensitive to parameters estimation. Using this logistic switch allows the Bayesian algorithm to deal with cases were the plant has bolted before its predicted bolting day, thus removing the constraints on the calculation of the time to reach peak N. It appears that the smooth transition is consistent with previous theoretical results and the case for flowering "at" peak N, dealt with in chapter 1, therefore consists in a particular case where ε is very small.

Continued vegetative growth has been long recognised as an issue in model predicting bang-bang strategies. In his seminal paper, Cohen (1971) identifies and solves this problem within his model by stating that the season length is stochastic hence the optimal plant has to have a transitional time with growth of both vegetative and reproductive tissues. In King and Roughgarden 1982, the problem is solved using multiple switches (reversion) between each compartment growth. Physiologically, the internal switch from a horizontal growth (rosette growth) to a vertical growth (stem elongation) may not be rooted at the time of bolting but later (Bernier *et al.* 1993). In the case of the peak N model with a smooth transition to flowering, data suggest that the plant is able to assess its nutritional status in order to modulate its allocation to reproduction. This transition does not consist in a hard switch but rather in a transition that ensures that the plant will possess the necessary structures to accumulate the nitrogen when uptake is maximised. The ability to detect nutritional status to trigger flowering has been studied

at the molecular level and is linked to the concentration of a particular sugar, trehalose-6-phosphate (Wahl *et al.* 2013).

The model needed a correction for the initial mass of the plant. When using the weighted seed mass to initialise growth, the model overestimated the growth rate (generally by selecting very high values of γ) and this later led to an overestimation of the plant reproductive production rate. Better estimation of the plant lifecycle was given by introducing a parameter that accounted for the difference between original seed mass and the initial mass that best suited the other parameter values. We suggest two phenomena that may explain why we had to include this correction. First, the initial growth environment, from germination until plants had two to four leaves, was different as the plastic foil was still in place. This was done to ensure a high germination rate and a maximal survival rate. This method proved successful as the germination rate was 100% and the death rate was 0% for this experiment. However, the presence of the plastic foil created conditions similar as a greenhouse with elevated temperature and humidity that may have provoked a higher growth rate than later in the experiment. The second possible explanation relies on plant physiology. It is likely that, as a plant gets bigger, the efficiency of photosynthetic activity decreases because of maintenance costs for example (Amthor 2000). As early plant may achieve near-exponential growth, the difference between seed size and plant size when we started collecting data may not correlate well.

Refinements of the model

Translocation improved the fit by diminishing the rate at which the plant builds its reproductive tissues, which is achieved through imperfect leaf mass to reproductive mass transfer. As it is generally admitted that nitrogen is more labile than carbon (Aerts *et al.* 1992, Schiltz *et al.*

2005), this finding may seem obvious. The low efficiency of translocation, 4% according to Filzbach, suggests that leaf senescence is rather driven by the loss of nitrogen than by the senescence of the whole leaf mass, carbon included. A case where only nitrogen is remobilised for reproductive structure was explored in the first chapter of this thesis where we suggest that yellowing of plant tissues without apparent size loss was linked to nitrogen remobilization (Jordi *et al.* 2000). It is difficult to relate this result to existing literature as leaf carbon translocation, when considered, is related to the photosynthetic activity (Earley *et al.* 2009). In Himelblau & Amasino (2001), the loss of carbon from the leaves is documented but the destination of this carbon is not assessed. Indeed, it appears difficult to estimate the fraction of senesced leaves that is translocated to seeds because it is virtually impossible to tell apart new carbon synthesised and directed to reproductive tissues from carbon removed from older tissues. We suggest it might be possible to track carbon of senesced leaf with an experiment using carbon isotopes only before bolting. This way, the new carbon photosynthesised would not be labelled and only senescing material would be tracked.

Increasing the complexity of the model by allowing different plant parts to have their own carbon-nitrogen ratios made sense in the light of the results from CHN analysis. The modification improves the fit mainly by allowing reproductive tissues to grow at a lowered rate compared to the global model, which overestimates it. The model with different C:N ratios was estimated to be the best model in terms of AIC (Table.4.4). Differences in rates of production are common assumptions in resource allocation models (e.g.: Lloyd 1984, Nord *et al.* 2011). When different ratios are considered, it is possible that the compartment requiring more nitrogen to grow is not the one that returns the biggest growth in terms of carbon production, which is different from the core equation from chapter 1 (Eqn.1.1-1.3). When all compartments have the

same C:N ratio, growth rate and nitrogen uptake maximisation are confounded and it is not possible to distinguish whether the plant optimises one or the other.

When running this model, we therefore considered that the plant was always nitrogen limited and would grow roots when needed even though it might not lead to the highest return in mass production. It is possible that a light-limited plants would solve this problem differently than a nitrogen-limited one (Cerdan and Chory 2003, Wilczek *et al.* 2009, Castro-Marin *et al.* 2011, Sugiura and Tateno 2011).

Ability to handle ecotype specifity

Setting ecotype-specific values for parameters improved the fit in the same proportion as for translocation and different C:N ratios (Table.4.4) but had the advantage to allow the prediction for bolting day as different physiological parameters for each ecotype allows for a wide range of possible bolting days (Figure.4.4). Ecotypes are selected in the wild according to a large variety of biotic and abiotic factors (MitchellOlds 1996, McKay *et al.* 2003, Zuest *et al.* 2011). In the context of our model, selection on improvement of the photosynthetic efficiency γ is linked with the time to flowering and may provide a simple explanation for the latitudinal variation of flowering time found in (Stinchcombe *et al.* 2004, Li *et al.* 2010). Selection on root uptake θ might be linked to water use efficiency (Pigliucci *et al.* 1995, McKay *et al.* 2003) and would also explain flowering time variation described by McKay 2003. With the experimental validation of the model carried out here in laboratory conditions, one can be confident with the peak N rule for flowering. At this point, the model should be tested in a wider variety of situations.

Postflowering strategies

In chapter 2, we used theoretical argument to select an "all-for-flower" strategy for flowering over four possible alternatives. Here, the data confirm this result: after the plant has initiated flowering, it uses newly produced photosynthates to grow reproductive tissues. This is shown by the great differences in AIC between translocative and photosynthetic strategies (Table.4.1). Furthermore, the comparison confirms that plant leaves senesce (a photosynthetic flowerer is worse than the "all-for-flower" strategy) and that the mass from senesced leaves is not directed towards roots (the photosynthetic competitor does not beat the "all-for-flower"). Our experiment confirms that the flowering behaviour predicted by the peak N model is followed by *Arabidopsis thaliana* and should hold for annual plants in general (Law 1979, Kozłowski 1992, Himelblau and Amasino 2001, Waters and Grusak 2008). However, perennial plants are likely to have evolved different post-flowering rules (Metcalf *et al.* 2008, Metcalf and Mitchell-Olds 2009) especially rules concerning translocation (Reekie and Bazzaz 1987).

Concluding remarks

The peak N model, provided minor changes, successfully predicts both quantitatively and qualitatively the life trajectories of *Arabidopsis thaliana* in normal laboratory conditions. Because the model outperforms alternative rules for flowering, we strongly suggest work to be carried out to examine the molecular basis of the behaviour our model predicts. Finally, as the peak N model also comes with a variety of theoretical results for biotic interactions, it is calling for tests in a broader range of conditions that might lead to new insights in our understanding of annual plants.

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General Conclusion

With the peak N hypothesis for flowering, we explored several aspects of the lifecycle of an annual plant. Inspired by the work of Iwasa (2000) dealing with growth rate optimisation coupled with the work of Chen & Reynolds (1997, 1996), we explored the conditions triggering flowering, assuming the plant lives by the most limiting resource in its environment: nitrogen. The model was explored theoretically in a systematic manner; it gave birth to a suite of interesting findings many of which now require experimental backup.

In the first place, we explored the effects of the physiological parameters defining growth for the plant. Resulting from this exploration, we hypothesise that a plant might choose to flower when it reaches the maximum of nitrogen uptake, peak N, as it marks the shift from a carbon-limited world to a nitrogen-limited one in which the plant can't escape a decreasing growth rate.

Because nitrogen is the factor set as limiting, we also explored how the way nitrogen is applied influences plant responses. The peak N model showed it was able to predict why a fertilised plant delays only slightly flowering, while reaching both a bigger vegetative and reproductive mass. In the same time, the model predicts earlier flowering in severely limiting nitrogen conditions such as reduced pot sizes.

The exploration continued when we included biotic interactions in the model on top of the already existing abiotic ones. Small alterations of the model allowed us to explore the effects of competition and herbivory on the plant lifecycle, with a main focus on flowering time and the final reproductive mass. We showed that a tragedy of the commons may not occur as a result of root competition, principally because the carrying capacity of an environment allows for only a limited final mass and therefore root overinvestment is unlikely to be optimal in a limiting

environment. Growth limitation or scarce resources was surprisingly not taken into account in the literature for the tragedy of the commons we had access to (Gersani *et al.* 2001, Semchenko *et al.* 2007, O'Brien *et al.* 2007).

We then explored the effects of herbivory on the plant flowering time, assuming the plant could not resist the aggression and only tolerate it. With this version of the model, we showed that the peak N hypothesis not only was able to ensure a near-optimal flowering timing in nitrogen-limited conditions but could also explain how a plant adapts its growth when facing herbivory of different kinds.

Finally, we gave our model some experimental validation. We used a set of ecotypes of the annual plant *Arabidopsis thaliana*, grown in a very limited nitrogen soil. We compared our hypothesis that the ecotypes differed in flowering time only because they had different physiological parameters and not because of a built-in flowering trigger such as an age- or a size-rule. Our experimental data showed that the peak N model had better predictive power at equivalent parameter numbers for the biomass prediction and a slightly worst predictive power for the timing of flowering. This strongly suggests annual plant would grow and flower according to their internal growth rate capability and therefore annual plants may be subjected to a very few set of laws for flowering that I describe as follows:

The three laws of flowering

1. Maximise vegetative growth

This is intended as an extension of Liebig's law that states the plant's growth is limited by the most limiting resource (Hooker 1917). As plant affects the environment it is growing in, it depletes resources needed to grow and will experience limitation both from the roots to gather

nitrogen and from the leaves to gather carbon. The work from Chen & Reynolds, limited to the vegetative part of the plant lifecycle pioneered the idea of co-limitation and optimal partitioning of growth. Model simulations within this thesis, as well as numerous other models (Kozłowski 1992, Iwasa 2000, Sugiura and Tateno 2011) predicts that maximising preflowering growth rate is correlated with higher fitness. This is backed up by numerous experimental articles (Lewis *et al.* 2003, Bolmgren and Cowan 2008, Todesco *et al.* 2010), and growth rate is commonly used as the measure of fitness involving comparisons among species or ecotypes (Koornneef *et al.* 1998, Du and Qi 2010, Zuest *et al.* 2011).

The only case, not explored here, when a decrease in growth rate may positively affect fitness is in the case of plant resistance. Defence has a cost in terms of growth rate (Purrington 2000, Paul-Victor *et al.* 2010). And, in the simple case, investment in defence is positive for fitness when it decreases the plants fitness less than the pertubation (e.g.: herbivory) causing the fitness decrease in the first place. Because of the wide variety of strategy plants display to resist perturbations (Herms and Mattson 1992), we decided to narrow the scope of our exploration and did not try to explore the tradeoffs between growth maximisation and resistance. Previous attempts to link these two traits led to an interesting literature, backed up with a wealth of data (Bryant *et al.* 1983, Coley *et al.* 1985, Lerdau and Coley 2002, Nitao *et al.* 2002, Endara and Coley 2011). Predicting resistance level in terms of disturbance level and intrinsic growth rate in the context of the peak N model certainly constitute an interesting avenue for future research.

2. Initiate flowering according to peak N

The second law of flowering, and the main focus of the present work, is the decision to flower. Growth optimisation is at the core of the flowering decision in the work of Cohen (1971, 1976)

and later Iwasa (Iwasa and Cohen 1989, Iwasa *et al.* 1996, Iwasa 2000). However, the distinction of our model is that its primary focus is not fitness; it is optimal partitioning through maximised growth. Put simply, the second law emerges from the first one. As a plant grows and its growth rate decreases, investing in reproductive mass ends up being the best allocation strategy in order to achieve a high mass. The peak N rule for flowering is flexible. It accommodates environmental conditions through its effects on growth rate to provide an optimal growth pattern through the season. But more importantly, the peak N rule is mathematically and conceptually simple. This, I believe, is key for an idea to spread beyond the fields of ecological modelling and be integrated in more complicated framework such as evolutionary game theory, quantitative genetics, or modelling at a larger scale.

The only alternative to flowering offered by our model is for the plant to become a plant only made of roots. While this strategy is obviously useless for an annual plant, there certainly lies some potential for the peak N model to give sensible predictions in the context of biannual or perennial plants. Extending our model to plants living longer than one growing season seems a natural next step.

3. Invest everything for reproduction

This last rule, tested theoretically in the second chapter and experimentally in the fourth chapter states that a flowering plant should reallocate leaf mass into reproductive mass after the initiation of flowering. However, this last rule is open to controversy as 1) our model does not account for survival and 2) our experimental model in chapter 4 allows for some vegetative growth past bolting. The peak N model predicts the existence of a natural program to remove unnecessary vegetative mass in order to maximise reproductive output. This program relies on the same

assessment of resource that drives the first two rules. In this sense, the three rules for flowering simply display the integrated lifecycle we suppose a plant is trying to achieve. The rule states, investing *for* reproduction; this does not equate investing *in* reproduction. Therefore the long-term evolutionary goal of the plant which is maximizing fitness is conserved and implied in this last rule.

The three laws of flowering merge together the physiological necessities of growth with the ecologically driven initiation of flowering that ultimately optimise the evolutionary necessity of fitness to be maximised through the process of natural selection. I called the three previous statements laws in a provocative manner, as I expect them to be challenged right away by experts in related fields. The mere modeling of how a plant *should* act is in no way a blueprint of how a plant *does* act. Therefore, these laws are to be seen as concurring to create a "simple but evocative model" of plant flowering (Box and Draper 1987). A model, I hope, will help biologists from various fields to integrate together their seemingly distant frameworks in a coherent manner.

What is next for the peak N hypothesis?

The peak N model offers a conceptual framework to understand the drivers of flowering and growth for an annual plant. So far, only the lifecycle in nitrogen limited conditions has received experimental validation. Competition, predation, and fertilisation have not received due attention in order to confirm the list of predictions given by the peak N model. A set of carefully designed experiment to systematically address our findings needs to take place. To test whether fertilisation affects the plant lifecycle as predicted, one would grow a set of ecotypes (differing in growth rate to assess the variability in physiological parameters) in different nitrogen level and

with different fertilisation rates.

In order to test the predictions for competition, another set of experiments would take place. A set of ecotypes, chosen 1) for their variability in seed mass and 2) their variability in flowering time would be grown together in a density gradient. The most important prediction to be confirmed is that competition forces plants to flower closer to peak N. It is also important to ensure no tragedy of the commons occurs.

Furthermore, the peak N model predicts changes in lifecycle according to variations in the type of herbivory. A set of experiments involving sap-sucking insects, leaf herbivores, root herbivores, and clipped plant parts would be necessary to test all the model predictions. To be thorough, the model tested for herbivory should be modified to include costs of resistance. Because a range of different resistance processes exist, this might have to be done on a one-by-one basis.

Finally, a natural-next step in research on the peak N model is to apply the model to a range of annual species. This will be the first step to test whether a peak N rule for flowering applies to a wide range of species. By gathering data of growth rate and flowering time from a large number of experiments, a systematic testing of the model can be accomplished with Filzbach. Given a large enough sample, this may allow to observe whether there are species cluster that possess similar physiological parameters for growth that would explain differences in flowering time, as is hinted in Ikram *et al.* (2012).

Besides experimental confirmations many other applications for our model can be thought of because the peak N model is designed as a building-brick for other problematics.

Molecular biologists using quantitative genetics sometimes co-localise flowering time genes with growth genes (Ungerer *et al.* 2003), nutrient-sensing genes (Castro Marín *et al.* 2011), or resistance genes (Wang *et al.* 2011) when they may in fact happen to be looking at genes

controlling for growth that ultimately affects resistance, nutrient uptake or flowering time. This would occur because growth is an integrated process. In a complex gene network, the clever addition of a relatively simple model such as peak N may allow to extend the predictive power of quantitative genetics models to ranges of ecological concerns such as competition or nitrogen depletion at the cost of little added complexity.

The use of fertiliser in agriculture was at the origin of the green revolution that allowed billions to escape starvation. In developed countries, the level of fertilisation has hit a maximum and crop yields have plateaued in the last 5 to 10 years (Aizen *et al.* 2008). One possible application of the peak N model could include the use of carefully designed fertilisation practices in order to maintain high yield with lower rate of fertilisation. This would not only save money but may also prevent environmental pollution (Lawlor 2002).

The process of growth is largely dependent on environmental conditions. Patches of nutrients centimeters apart may cause large mass disparities in related individuals (Huston and Deangelis 1994). Changes in CO2 concentration, light quality and soil nutrient stoichiometry are likely to affect an annual plant's lifecycle (Vrede *et al.* 2012). Although the peak N model provides quantitative answers in a simple two-resources world, the acquisition of very precise data on the plant nutrient uptake –not only nitrogen but also phosphorus and potassium- together with aerial carbon, light quality and quantity could lead to the creation of an even more precise model. The peak of growth that is mirrored by peak N in our model could be precisely defined as the balance of all environmental factors that influence growth rate. This will lead the way toward both a deeper understanding of plants lifecycle and more practical application of ecological modelling for agricultural or preservation practices.

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somewhere on the planet! Xue Fei, my memories of the institute would be so different without our F5's! You taught me that being impulsive was also a positive thing (planning a trip to China in 5 minutes, really?). But more importantly, you took my hand and explained to me what being Chinese in the 21st century was about. 你很好老师. **谢谢**. Andy, you were always – metaphorically – standing there in the back, looking at progress I made and helping when necessary. When funding ran out, you were there to help me finish my PhD in an adequate financial position. This will never be forgotten. Thank you very much for the nice dinners and the awesome retreats with your group and the L-team. I would also like to thank many members of Andy's group: Juliette, tu as été d'une grande aide lorsque nous nous sommes installés et tu es une personne vachement cool, j'espère que nos chemins continueront de se croiser. Dzaeman, you may be the most socially-skilled person I know. It was great to follow your lead in many instances, be it an improvised Halloween choreography or a night out in London. Yan, j'ai dès le départ admiré ta manière d'aborder la science. Tu m'as aidé à la fois à mieux comprendre les suisses et ce que je devais faire en tant que scientifique. Merci. As mentioned earlier, I shared office with a bunch of crazy people that made the institute not just a working place, but an awesome place to be. But this atmosphere would not exist without you, Bernhard Schmid. You successfully work to make this institute more than a place where we do science, but a place where everyone enjoy doing science! Thank you for that, and the dinners at your place, and the two Sino-Swiss Summer Schools and thank you for being the professor I can only dream to one day become. Just a word to all the people: Ali, you contributed a lot to the nice atmosphere in 13H26. You DO speak loud and a lot, that's what makes you adorable. Matteo, reste toi-même et accepte la part de France en toi. Janielle, in the end, you're my academic sister, door will always be open for you. Sam Wuest, thanks for the Zusammenfassung and for being an incredibly

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